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Cátedra de Anatomía Patológica, Facultad de Odontología, Universidad de Buenos Aires.
M.T. de Alvear 2142 (C1122AAH) Buenos Aires, Argentina.
http://www.actaodontologicalat.com/contacto.html
actaodontologicalat@gmail.com

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Association between dental caries and sociodemographic factors in children aged 5-12 years

Elmer Jehovani Portillo-Sorto

Universidad Evangélica de El Salvador, Facultad de Odontología, Observatorio de Salud Bucodental, San Salvador, El Salvador.

ABSTRACT

Dental caries is a global public health issue, particularly in children, and is influenced by sociodemographic conditions. ***Aim:*** *To assess the association between dental caries and sociodemographic factors in children aged 5-12 years in Victoria, Cabañas, El Salvador.* ***Materials and Method:*** *A cross-sectional, correlational study was conducted among 137 children (48.2% boys, 51.8% girls), measuring dental caries using DMFT and dmft indices, and oral hygiene using the O'Leary Plaque Index. Sociodemographic data were obtained through caregiver questionnaires.* ***Results:*** *Caries prevalence was 63.5%. DMFT increased with age (highest in 10–12-year-old girls: mean = 5.44), while dmft declined (lowest in 12-year-old girls: mean = 0.56). Most children (98.5%) had poor oral hygiene. A weak but significant negative correlation was found between income and dmft (ρ = -0.189; p = 0.027). No other significant association was observed between caries indices and maternal education, income, or plaque index.* ***Conclusions:*** *Caries patterns differed significantly according to age and sex. Despite the high prevalence, most sociodemographic variables showed no strong statistical association with caries. Preventive interventions should focus on age- and sex-specific risks.*

Keywords: *dental caries - dental plaque index - children - educational status - income*

Relación entre el diagnóstico de caries dental y los factores sociodemográficos en niños de 5 a 12 años

RESUMEN

La caries dental infantil representa un problema de salud pública global, influenciado por factores sociodemográficos. ***Objetivo:*** *Evaluar la relación entre caries dental y factores sociodemográficos en niños de 5 a 12 años en Victoria, Cabañas, El Salvador.* ***Materiales y Método:*** *Estudio transversal, correlacional, con 137 niños (48.2% niños, 51.8% niñas). Se emplearon los índices CPOD y ceod para medir caries, y el índice de placa de O'Leary para higiene oral. Se recolectaron datos sociodemográficos mediante cuestionarios.* ***Resultados:*** *La prevalencia de caries fue del 63.5%. El CPOD aumentó con la edad (mayor en niñas de 10–12 años: media = 5.44), mientras que el ceod disminuyó (menor en niñas de 12 años: media = 0.56). El 98.5% presentó mala higiene bucal. Se halló una correlación negativa débil pero significativa entre ingresos y ceod (ρ = -0.189; p = 0.027). No se encontraron asociaciones significativas con educación materna o el índice de placa.* ***Conclusiones:*** *Los índices de caries variaron significativamente por edad y sexo. Aunque la prevalencia fue alta, los factores socioeconómicos evaluados no mostraron asociación significativa. Se recomienda implementar estrategias preventivas enfocadas según edad y sexo.*

Palabras clave: *caries dental - índice de placa dental - niños - nivel educativo - ingresos*

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Corresponding Author:
Elmer Jehovani Portillo-Sorto
jehovaniportillo@icloud.com

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INTRODUCTION

Oral health is a global public health concern, particularly among vulnerable pediatric populations in low- and middle-income countries, where economic and social inequalities exacerbate disparities in access to care[1]. Despite being largely preventable, dental caries is the most prevalent chronic condition in childhood, impacting overall well-being and quality of life[2].

According to the 2022 Global Oral Health Status Report from the World Health Organization (WHO), 514 million children suffer from caries in primary teeth and over 2 billion people have untreated caries in permanent teeth[3]. Urbanization, high consumption of sugary foods, limited fluoride exposure, inadequate oral hygiene practices, and insufficient access to dental services contribute significantly to this burden[4-5].

Research from around the world has consistently demonstrated a strong association between sociodemographic determinants and caries prevalence in children. For example, studies from Japan, Brazil and Latin America have shown that low maternal education, household income, and socioeconomic status are associated with higher prevalence of dental caries[6-8]. Similarly, Vasireddy et al. found that children in the lowest income bracket in the U.S. were more than twice as likely to develop caries compared to those from higher-income families[9]. In Latin America, Ojeda and Villavicencio identified income as a barrier to access to dental care[10]. Recent evidence from Syria and Ethiopia highlights that school type, household size, parental education, and dietary behaviors are significantly associated with caries experience[11-12]. A recent national study in Peru reported that 76.2% of children aged 3 to 5 had dental caries[13]. In China, research in 2024 showed that 97% of caries in preschoolers were untreated, underscoring limited access to early treatment[14]. All these studies highlight the importance of identifying vulnerable populations in order to plan preventive interventions.

Despite the growing body of international literature, localized data are essential to guide effective community-based public health strategies. In El Salvador, data on dental caries and their association with social determinants are scarce. This study addresses this gap by examining the association between sociodemographic factors and dental caries in children aged 5-12 in Victoria, Cabañas, a region with limited access to healthcare and high poverty levels.

This study employed validated caries indices DMFT (Decayed, Missing, and Filled Teeth for permanent dentition) and dmft (for primary dentition), as well as a structured caregiver questionnaire to explore how age, sex, maternal education and family income influence oral health status in children.

Research question: What is the association between dental caries diagnosis and sociodemographic factors such as age, sex, maternal education and family income among children aged 5 to 12 years in Victoria, Cabañas, El Salvador?

Hypothesis: Sociodemographic factors, including age, sex, maternal education level and family income significantly influence the prevalence and severity of dental caries in children aged 5 to 12 years in Victoria, Cabañas, El Salvador.

Null hypothesis (H_0): There is no significant association between dental caries diagnosis and the sociodemographic factors under study.

Alternative hypothesis (H_1): There is a significant association between dental caries diagnosis and at least one of the sociodemographic factors (age, sex, maternal education or family income).

MATERIALS AND METHOD

Ethical considerations

Participants' rights were protected through informed consent signed by parents or guardians, verbal assent from children, voluntary participation, and guaranteed confidentiality. The study was approved by the Health Research Ethics Committee (ACTA No. 339).

Study design

This was a quantitative, cross-sectional, observational, correlational study conducted in 2023 in Victoria, Cabañas, El Salvador. A total of 137 children aged 5 to 12 years (66 boys and 71 girls) participated. A non-probabilistic convenience sampling method was used, justified by logistical limitations in rural areas and the inability to construct a comprehensive sampling frame within the study timeframe.

Inclusion and exclusion criteria

Children aged 5 to 12 years residing in Victoria, Cabañas, El Salvador, whose parents or guardians

signed the informed consent, were included. Exclusion criteria were presence of systemic diseases, ongoing orthodontic treatment, or absence on the day of the dental evaluation.

Clinical assessment and data collection

Dental caries were diagnosed using the DMFT (Decayed, Missing, and Filled Teeth) and dmft indices for permanent and primary dentition, respectively, following the World Health Organization guidelines[15]. Three trained, calibrated examiners performed the clinical assessments. Dental plaque was assessed using the O'Leary Plaque Index[16] by four trained observers, applying a plaque disclosing agent to smooth tooth surfaces.

Instruments

The instruments included standardized clinical records, dental charts, plaque index forms, and a validated questionnaire designed by experts in pediatric dentistry, public health and research. The questionnaire recorded the following sociodemographic information: age (in years), sex (male/female), maternal education level (no schooling, primary, secondary, higher), and monthly household income (<$100, $100-199, $200-299, $300-499 USD).

Quality control and bias reduction

Calibration among observers was conducted before data collection. Kappa coefficients showed high interobserver agreement: 0.852 and 0.855 for caries diagnosis; 0.855 and 0.719 for plaque evaluation. To reduce information bias, surveys were administered by trained personnel. Selection bias was minimized by including all children who met inclusion criteria and were available during the evaluation period.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 23[17]. The Kolmogorov-Smirnov test was applied to assess the normality of the data, revealing non-normal distribution for most variables. Consequently, non-parametric tests were used: Mann-Whitney U for two-group comparisons, Kruskal-Wallis for multiple groups, and Spearman's rank correlation for ordinal variables. Descriptive statistics included frequency distributions, measures of central tendency, and cross-tabulations to assess the distribution of caries and plaque indices by age and sex.

RESULTS

Descriptive Analysis

DMFT, dmft, and plaque index values according to age and sex in children aged 5 to 12 years

A progressive increase in the DMFT index was observed with age, particularly among girls, whereas the dmft index decreased in older children, showing higher prevalence among those under 7 years of age. The plaque index remained high across all age groups, with worse outcomes in girls, reflecting a generalized pattern of poor oral hygiene (Table 1).

The prevalence of caries in permanent teeth was 63.5%, with a Significant Caries Index (SCI) of 6.82, indicating higher rates in girls. The average plaque index was 74.97%, and the 75th percentile revealed

Table 1. Mean, median, and 75th percentile values of DMFT, dmft and plaque index according to age and sex (n = 137)

Age Group	Sex	Mean DMFT	Mean dmft	Mean PI (%)	Median DMFT	Median dmft	Median PI (%)	75th Perc. DMFT	75th Perc. dmft	75th Perc. PI (%)
5-6	Male	0.36	8.05	65.18	0	7.5	65.18	0.25	11.25	81.34
5-6	Female	0.43	7.93	77.71	0	8.5	86.16	0.25	10.25	90.31
7-9	Male	1.91	5.22	75.72	1	5	83.7	3	8	90
7-9	Female	2.43	6.1	80.57	2	6	89.13	3.5	9	99.38
10-11	Male	3.85	2.15	78.17	4	1	83.33	4.5	3.5	96.21
10-11	Female	5.44	1.41	82.75	4	1	87.5	8	2	95.65
12	Male	3.13	1	67.47	3	0	78.82	5.5	1.5	89.9
12	Female	4.33	0.56	71.26	4	0	78.13	5.5	1.5	96.88

Note. DMFT = decayed, missing and filled teeth in permanent dentition; dmft = same index for primary teeth. PI = plaque index based on stained surfaces. Data obtained from clinical assessments of 137 children in Victoria, Cabañas (2023).

Table 2. Distribution of DMFT index categories according to age group and sex in children aged 5 to 12 years (n = 137)

Age Group	Sex	Very Low (0–1.1)	Low (1.2–2.6)	Moderate (2.7–4.4)	High (4.5–6.5)	Very High (≥6.6)	Total
5-6	Male	20 (30.3%)	1 (1.5%)	1 (1.5%)	0 (0.0%)	0 (0.0%)	22
7-9	Male	12 (18.2%)	3 (4.5%)	5 (7.6%)	2 (3.0%)	1 (1.5%)	23
10-11	Male	1 (1.5%)	3 (4.5%)	6 (9.1%)	2 (3.0%)	1 (1.5%)	13
12	Male	2 (3.0%)	1 (1.5%)	3 (4.5%)	2 (3.0%)	0 (0.0%)	8
	Total	**35 (53.0%)**	**8 (12.1%)**	**15 (22.7%)**	**6 (9.1%)**	**2 (3.0%)**	**66**
5-6	Female	13 (18.3%)	0 (0.0%)	1 (1.4%)	0 (0.0%)	0 (0.0%)	14
7-9	Female	8 (11.3%)	5 (7.0%)	5 (7.0%)	2 (2.8%)	1 (1.4%)	21
10-11	Female	5 (7.0%)	2 (2.8%)	7 (9.9%)	5 (7.0%)	8 (11.3%)	27
12	Female	1 (1.4%)	1 (1.4%)	3 (4.2%)	3 (4.2%)	1 (1.4%)	9
	Total	**27 (38.0%)**	**8 (11.3%)**	**16 (22.5%)**	**10 (14.1%)**	**10 (14.1%)**	**71**
	Overall Total	**62 (45.3%)**	**16 (11.7%)**	**31 (22.6%)**	**16 (11.7%)**	**12 (8.8%)**	**137**

Note. DMFT = Decayed, Missing and Filled Teeth index in permanent dentition. Categories were classified following WHO guidelines for epidemiological comparisons. Percentages refer to the proportion of the total sample within each sex and age group.

a subgroup with alarmingly high values across all indices, suggesting the need for specialized dental care.

Association among age, sex and DMFT index

In the 5-6-year age group, 24.1% of the children had very low DMFT indices. In the 7-9-year group, 14.6% maintained low DMFT values, although 1.5% reached very high levels. Among children aged 10–12 years, the proportion of moderate to very high DMFT indices increased markedly, especially in girls, indicating a progression of caries in permanent teeth with age and sex-related differences in prevalence (Table 2).

Association among age, sex and dmft index

In the 5–6-year age group, a high proportion of children had very high dmft scores, with 21.2% of boys and 14.1% of girls being included in this category. Similarly elevated values were observed in the 7–9-year age group, although a slight reduction was noted. Among older children, especially 12-year-old girls, the dmft index declined progressively, reflecting the natural shedding of primary teeth and a reduced impact of caries on deciduous dentition at older ages (Table 3).

Association between DMFT index and mother's education level

Children whose mothers had only received primary education exhibited the highest proportion of very low DMFT indices (31.4%). However, paradoxically, this group also concentrated the most cases of caries, indicating a high overall prevalence of caries despite the distribution. A downward trend in DMFT values was observed as maternal education level increased, suggesting that higher maternal education may have a protective influence on permanent dentition. Nonetheless, statistical analysis did not reveal a significant correlation between maternal education and DMFT index (Rho = 0.070, p = 0.418). This may be influenced by the homogeneity of the study population or by uncontrolled confounding variables (Table 4).

Association between dmft index and mother's education level

Children whose mothers had no schooling or only basic education exhibited higher dmft indices. Specifically, 22.6% of the children in the primary education group had a "very high" dmft index, indicating greater vulnerability to dental caries in primary dentition when maternal education is limited. In contrast, children whose mothers had secondary, or university-level education tended to have lower dmft values. However, the statistical analysis revealed no significant correlation between maternal education level and the dmft index (Rho = 0.070; p = 0.418). This lack of association could be attributed to the limited variability in educational

Table 3. Distribution of dmft index categories according to age group and sex in children aged 5 to 12 years

Age Group	Sex	Very Low (0–1.1)	Low (1.2–2.6)	Moderate (2.7–4.4)	High (4.5–6.5)	Very High (≥6.6)	Total
5-6	Male	1 (1.5%)	2 (3.0%)	0 (0.0%)	5 (7.6%)	14 (21.2%)	22
7-9	Male	5 (7.6%)	1 (1.5%)	5 (7.6%)	2 (3.0%)	10 (15.2%)	23
10-11	Male	7 (10.6%)	0 (0.0%)	4 (6.1%)	2 (3.0%)	0 (0.0%)	13
12	Male	7 (10.6%)	1 (1.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8
	Total	**20 (30.3%)**	**4 (6.1%)**	**9 (13.6%)**	**9 (13.6%)**	**24 (36.4%)**	**66**
5-6	Female	1 (1.4%)	0 (0.0%)	1 (1.4%)	2 (2.8%)	10 (14.1%)	14
7-9	Female	4 (5.6%)	0 (0.0%)	1 (1.4%)	6 (8.5%)	10 (14.1%)	21
10-11	Female	16 (22.5%)	5 (7.0%)	4 (5.6%)	2 (2.8%)	0 (0.0%)	27
12	Female	7 (9.9%)	2 (2.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	9
	Total	**28 (39.4%)**	**7 (9.9%)**	**6 (8.5%)**	**10 (14.1%)**	**20 (28.2%)**	**71**
	Overall Total	**48 (35.0%)**	**11 (8.0%)**	**15 (10.9%)**	**19 (13.9%)**	**44 (32.1%)**	**137**

Note. dmft = decayed, missing and filled teeth in primary dentition. Classification follows WHO criteria. Percentages represent the proportion of the total sample in each subgroup.

Table 4. Distribution of DMFT index categories according to mother's education level (n = 137)

DMFT Category	No Schooling	Early Childhood Education	Primary Education	Secondary Education	Higher Education	Total
Very Low (0–1.1)	5 (3.6%)	1 (0.7%)	43 (31.4%)	11 (8.0%)	2 (1.5%)	62
Low (1.2–2.6)	0 (0.0%)	1 (0.7%)	7 (5.1%)	8 (5.8%)	0 (0.0%)	16
Moderate (2.7–4.4)	2 (1.5%)	3 (2.2%)	19 (13.9%)	5 (3.6%)	2 (1.5%)	31
High (4.5–6.5)	0 (0.0%)	1 (0.7%)	12 (8.8%)	3 (2.2%)	0 (0.0%)	16
Very High (≥6.6)	1 (0.7%)	0 (0.0%)	5 (3.6%)	5 (3.6%)	1 (0.7%)	12
Total	**8 (5.8%)**	**6 (4.4%)**	**86 (62.8%)**	**32 (23.4%)**	**5 (3.6%)**	**137**

Note. DMFT = decayed, missing and filled teeth in permanent dentition. Education levels were self-reported by parents and categorized according to national standards. Percentages represent proportions relative to the total sample.

levels within the sample or to the influence of other unmeasured factors such as access to dental care or hygiene habits supervised by caregivers (Table 5).

Association between DMFT index and family income

Children from families earning less than $100 per month showed a higher proportion of moderate

Table 5. Distribution of dmft index categories according to mother's education level (n = 137)

dmft Category	No Schooling	Early Childhood Education	Primary Education	Secondary Education	Higher Education	Total
Very Low (0–1.1)	1 (0.7%)	1 (0.7%)	28 (20.4%)	15 (10.9%)	3 (2.2%)	48
Low (1.2–2.6)	0 (0.0%)	2 (1.5%)	6 (4.4%)	3 (2.2%)	0 (0.0%)	11
Moderate (2.7–4.4)	2 (1.5%)	0 (0.0%)	11 (8.0%)	2 (1.5%)	0 (0.0%)	15
High (4.5–6.5)	2 (1.5%)	1 (0.7%)	10 (7.3%)	6 (4.4%)	0 (0.0%)	19
Very High (≥6.6)	3 (2.2%)	2 (1.5%)	31 (22.6%)	6 (4.4%)	2 (1.5%)	44
Total	**8 (5.8%)**	**6 (4.4%)**	**86 (62.8%)**	**32 (23.4%)**	**5 (3.6%)**	**137**

Note. dmft = decayed, missing and filled teeth in primary dentition. The data reflects maternal education levels and their association with caries prevalence in children. Percentages indicate the proportion of each group relative to the full sample.

(22.6%) and very high (8.8%) DMFT indices, indicating a greater burden of caries in permanent dentition among lower-income groups. In contrast, children from families with monthly incomes between $300 and $500 demonstrated significantly better oral health, with 45.3% presenting a "very low" DMFT index. Nevertheless, Spearman's correlation analysis showed no statistically significant association between family income and DMFT index (Rho = 0.030; p = 0.730). This lack of significance may reflect the relative homogeneity of the sample or the influence of confounding factors such as dietary habits or previous access to preventive dental care (Table 6).

Table 6. Distribution of DMFT index categories according to family monthly income (n = 137)

DMFT Category	< $100.00	$100.00–$199.99	$200.00–$299.99	$300.00–$499.99	Total
Very Low (0–1.1)	27 (19.7%)	20 (14.6%)	10 (7.3%)	5 (3.6%)	62
Low (1.2–2.6)	8 (5.8%)	3 (2.2%)	5 (3.6%)	0 (0.0%)	16
Moderate (2.7–4.4)	13 (9.5%)	11 (8.0%)	4 (2.9%)	3 (2.2%)	31
High (4.5–6.5)	6 (4.4%)	2 (1.5%)	2 (1.5%)	6 (4.4%)	16
Very High (≥6.6)	7 (5.1%)	3 (2.2%)	2 (1.5%)	0 (0.0%)	12
Total	**61 (44.5%)**	**39 (28.5%)**	**23 (16.8%)**	**14 (10.2%)**	**137**

Note. DMFT = decayed, missing and filled teeth in permanent dentition. This table shows how caries severity varies according to household monthly income. The "Very Low" category was most prevalent among children from families earning $300.00–$499.99.

Association between dmft index and family income

The cross-tabulation between the dmft index and monthly income ranges showed that children from families earning less than $100 per month exhibited higher rates of "very high" (18.2%) and "high" (6.6%) dmft indices, indicating a greater prevalence of caries in primary teeth. In contrast, children from families with incomes between $300 and $500 showed lower caries levels, with a greater proportion falling into the "very low" category (5.1%).

Overall, 35.0% of the children had a "very low" dmft index, while 32.1% showed a "very high" value, suggesting a polarized distribution of caries in primary dentition. Spearman's correlation revealed a weak but statistically significant negative association between family income and the dmft index (Rho = –0.189; p = 0.027), indicating that lower income levels are associated with higher caries prevalence in deciduous teeth. These findings underscore the importance of implementing targeted preventive strategies for low-income populations (Table 7).

Table 7. Distribution of dmft index categories according to family monthly income (n = 137)

dmft Category	< $100.00	$100.00–$199.99	$200.00–$299.99	$300.00–$499.99	Total
Very Low (0–1.1)	15 (10.9%)	18 (13.1%)	8 (5.8%)	7 (5.1%)	48
Low (1.2–2.6)	3 (2.2%)	3 (2.2%)	2 (1.5%)	3 (2.2%)	11
Moderate (2.7–4.4)	9 (6.6%)	3 (2.2%)	3 (2.2%)	0 (0.0%)	15
High (4.5–6.5)	9 (6.6%)	6 (4.4%)	3 (2.2%)	1 (0.7%)	19
Very High (≥6.6)	25 (18.2%)	9 (6.6%)	7 (5.1%)	3 (2.2%)	44
Total	**61 (44.5%)**	**39 (28.5%)**	**23 (16.8%)**	**14 (10.2%)**	**137**

Note. dmft = decayed, missing and filled teeth in primary dentition. The data indicates a higher prevalence of severe caries in children from lower-income families, particularly those earning less than $100.00 per month.

Association between DMFT index and plaque index

Analysis of the association between the DMFT index and plaque index revealed that most children (98.5%) had poor oral hygiene, as defined by plaque coverage exceeding 30%. Among these children, although 45.3% presented "very low" DMFT values, a considerable proportion also exhibited moderate (22.6%), high (11.7%) and very high (8.8%) DMFT indices (Table 8).

These results indicate a heterogeneous distribution

Table 8. Distribution of DMFT index categories according to oral hygiene (plaque index) in children aged 5–12 years (n = 137)

DMFT Category	Good (<20%)	Acceptable (20%–30%)	Poor (>30%)	Total
Very Low (0–1.1)	0 (0.0%)	0 (0.0%)	62 (45.3%)	62
Low (1.2–2.6)	0 (0.0%)	0 (0.0%)	16 (11.7%)	16
Moderate (2.7–4.4)	0 (0.0%)	0 (0.0%)	31 (22.6%)	31
High (4.5–6.5)	1 (0.7%)	1 (0.7%)	14 (10.2%)	16
Very High (≥6.6)	0 (0.0%)	0 (0.0%)	12 (8.8%)	12
Total	**1 (0.7%)**	**1 (0.7%)**	**135 (98.5%)**	**137**

Note. DMFT = decayed, missing and filled permanent teeth. Poor oral hygiene was nearly universal (98.5%), with the highest caries burden found in children with plaque indices above 30%.

of caries severity despite uniformly poor hygiene conditions. The absence of a statistically significant correlation between DMFT and the plaque index (Rho = 0.148; $p = 0.084$) suggests that while plaque accumulation is widespread, it may not fully explain the variation in caries prevalence in permanent dentition. Additional factors such as diet, fluoride exposure and previous access to dental care may contribute to this disparity and should be further explored in future research.

Association between dmft index and plaque index

Among children with poor oral hygiene (defined as a plaque index greater than 30%), 32.1% exhibited a "very high" dmft index, indicating high prevalence of dental caries in primary teeth within this group. However, 33.6% of these children had a "very low" dmft index despite poor hygiene (Table 9).

These findings suggest that while oral hygiene is an important factor, it is not the sole determinant of caries prevalence in primary dentition. The lack

Table 9. Distribution of dmft Index Categories According to Oral Hygiene (Plaque Index) in Children Aged 5–12 Years (n = 137)

dmft Category	Good (<20%)	Acceptable (20%–30%)	Poor (>30%)	Total
Very Low (0–1.1)	1 (0.7%)	1 (0.7%)	46 (33.6%)	48
Low (1.2–2.6)	0 (0.0%)	0 (0.0%)	11 (8.0%)	11
Moderate (2.7–4.4)	0 (0.0%)	0 (0.0%)	15 (10.9%)	15
High (4.5–6.5)	0 (0.0%)	0 (0.0%)	19 (13.9%)	19
Very High (≥6.6)	0 (0.0%)	0 (0.0%)	44 (32.1%)	44
Total	**1 (0.7%)**	**1 (0.7%)**	**135 (98.5%)**	**137**

Note. dmft = decayed, missing and filled primary teeth. Although most children had poor hygiene (98.5%), nearly one-third (33.6%) had a low dmft index, indicating that hygiene alone does not explain caries patterns in primary teeth.

of a significant correlation between plaque index and dmft (Rho = –0.005; $p = 0.953$) reinforces this interpretation. Other variables such as sugar intake frequency, fluoride use, caregivers' educational level, and access to dental care probably play a critical role in shaping children's caries experience.

Inferential Analysis

Statistical analysis of DMFT and dmft indices according to sociodemographic variables and plaque index.

This inferential analysis explored how the DMFT and dmft indices vary in relation to key sociodemographic factors, including sex, age, maternal education level, family income and plaque index (PI) (Table 10).

- **Sex**: The Mann-Whitney U test revealed a statistically significant difference in DMFT scores according to sex (U = 1813.000; $p = 0.019$), with girls showing higher caries experience in permanent teeth. In contrast, differences in dmft scores were not significant (U = 1977.000; p =

Table 10. Inferential statistical analysis of DMFT and dmft indices according to sociodemographic and clinical variables in children aged 5–12 years (n = 137)

Analysis	Dependent Variable	Independent Variable	Test Used	Statistic	p-value	Interpretation
Mann–Whitney U Test	DMFT	Sex	U	1813	0.019	Significant difference: girls had higher DMFT than boys.
Mann–Whitney U Test	dmft	Sex	U	1977	0.112	No significant difference in dmft according to sex.
Kruskal–Wallis Test	DMFT	Age group	H	52.588	<0.001	Significant increase in DMFT with age.
Kruskal–Wallis Test	dmft	Age group	H	65.686	<0.001	Significant decrease in dmft with age.
Spearman's Correlation	DMFT	Mother's education level	Rho	0.07	0.418	No significant correlation.
Spearman's Correlation	dmft	Mother's education level	Rho	0.07	0.418	No significant correlation.
Spearman's Correlation	DMFT	Monthly family income	Rho	0.03	0.73	No significant correlation.
Spearman's Correlation	dmft	Monthly family income	Rho	–0.189	0.027	Weak but significant negative correlation: lower income linked to higher dmft.
Spearman's Correlation	DMFT	Plaque Index	Rho	0.148	0.084	No significant correlation.
Spearman's Correlation	dmft	Plaque Index	Rho	–0.005	0.953	No significant correlation.

Note. DMFT = decayed, missing and filled permanent teeth; dmft = primary teeth; p-values < 0.05 considered statistically significant. The results indicate associations of sex and age with DMFT, and a weak inverse association between income and dmft.

0.112), suggesting that sex did not play a major role in caries prevalence in primary teeth.

- **Age**: The Kruskal-Wallis test indicated statistically significant differences in both DMFT (H = 52.588; $p < 0.001$) and dmft (H = 65.686; $p < 0.001$) across age groups. These results reflect the expected pattern: DMFT increases with age, while dmft decreases, consistently with the natural shift from primary to permanent dentition.
- **Maternal education level**: Spearman's correlation showed no statistically significant association between the mother's level of education and either the DMFT (Rho = 0.070; p = 0.418) or the dmft index (Rho = 0.070; p = 0.418). These findings suggest that, within this sample, maternal education was not a key determinant of caries status.
- **Family income**: The correlation between family income and DMFT was not significant (Rho = 0.030; p = 0.730). However, a weak but statistically significant inverse correlation was observed between family income and dmft (Rho = –0.189; p = 0.027), indicating that children from lower-income families were more likely to present higher caries prevalence in their primary teeth.
- **Plaque index**: No significant correlation was found between the plaque index and DMFT (Rho = 0.148; p = 0.084) or between PI and dmft (Rho = –0.005; p = 0.953). Thus, in this sample, oral hygiene measured by plaque accumulation did not have a direct statistical association with caries prevalence in either dentition.

Summary of Findings

- **DMFT index** was significantly influenced by **sex** and **age**, with higher values in girls and older children.
- **dmft index** was significantly affected by **age** and showed a weak but meaningful association with **family income**.
- **Maternal education** and **plaque index** were not significantly associated with either the DMFT or dmft caries index.

- The lack of statistical significance in some variables (e.g., DMFT vs. income) may be explained by sample homogeneity or unmeasured confounders.

These results underscore the importance of **targeted interventions** for specific age and socioeconomic groups. Future studies should consider additional determinants such as dietary habits, fluoride exposure, and access to dental services to better understand and reduce caries risk in children.

DISCUSSION

The results of this study are consistent with global trends described by the World Health Organization, which report a high prevalence of dental caries, particularly in low- and middle-income countries. A substantial burden of caries was identified in both primary and permanent dentition, aligning with WHO estimates indicating that 514 million children are affected by caries in primary teeth and more than 2 billion people have untreated caries in permanent teeth.[3] Factors such as urbanization, limited fluoride exposure, and restricted access to dental care continue to be major contributors to this burden.[3]

Socioeconomic factors and caries prevalence

Our findings are consistent with studies highlighting the influence of parental income and educational level on dental caries prevalence.[4-8] However, no statistically significant associations were observed between these variables and caries indices in the present study, which may be partially explained by the relative homogeneity of the study population. In contrast, international reports, including the 2024 CDC Oral Health Surveillance Report[18] and analyses based on U.S. national data[19], have documented strong associations between low income, gaps in health insurance coverage, and higher prevalence of untreated dental caries.

Regional differences in caries prevalence

This study observed an increase in caries prevalence with age. Fernández-de-Quezada et al.[20] reported similar patterns among 12-year-old children in El Salvador, while Danke et al.[21] documented declining trends in caries-free prevalence among younger children in Chile. Additionally, high caries rates reported in Syria[11] and Ethiopia[12] support the notion that regional and contextual factors play a significant role in shaping the burden of dental caries across populations.

Maternal education and caries prevalence

Although no statistically significant association between maternal education level and caries prevalence was observed in the present study, previous research has consistently demonstrated its relevance. Studies by Kumar et al.[5], Kato et al.[6], Lopes et al.[7], and Carvalho et al.[8] have shown that higher maternal education levels are associated with a reduced risk of dental caries in children. The absence of such an association in this study may be partly explained by limited variability in educational attainment among participants, suggesting that the influence of maternal education may be less evident in relatively homogeneous populations, despite its recognized importance in other settings.

Parental income and caries prevalence

The pattern observed in this study—namely, a higher prevalence of dental caries among children from lower-income families—is consistent with population-based evidence reported in national oral health surveys and epidemiological research. In Spain, data from the 2020 Oral Health Survey revealed persistent social gradients in caries experience among children, with a higher burden observed in socially disadvantaged groups.[22] Similar patterns have been documented in local studies conducted in low socioeconomic settings.[23]

Furthermore, de Lucena et al.[24] and Folayan et al.[25] demonstrated that structural socioeconomic inequalities significantly influence children's oral health, although the magnitude of these effects may vary across contexts. In Peru, a national study published in 2024 reported that 76.2 % of children aged 3 to 5 years presented dental caries, underscoring the need for public programs targeting vulnerable populations.[13]

Likewise, an ecological analysis conducted across European Union countries found that the burden of early childhood caries was significantly associated with structural socioeconomic indicators, including poverty risk, gross domestic product per capita, urbanization, and sugar consumption.[26] These findings highlight that socioeconomic inequities act as upstream determinants of caries burden, even within highly developed regions.

Complementarily, evidence from Mexico indicates that socioeconomic and contextual factors significantly influence oral health–related behaviors among schoolchildren. A multi-site study conducted

in several Mexican cities reported significant associations between socioeconomic position and toothbrushing frequency in children aged 6 to 12 years, highlighting the role of family environments and broader structural conditions in shaping daily oral hygiene practices.[27] These findings support the concept that socioeconomic inequities operate as upstream determinants of caries risk and reinforce the need for comprehensive interventions that address both individual behaviors and the social context in which they occur.

Similarly**,** a study conducted in Chile reported a higher prevalence of dental caries among schoolchildren from more disadvantaged social backgrounds, particularly among those attending public schools and with limited access to dental services.[21] This pattern is consistent with the findings of the present study and further underscores the influence of social determinants and institutional settings on children's oral health outcomes.

Oral hygiene and dental caries

Although the statistical correlations observed in the present study were weak, from a clinical perspective an association between oral hygiene–related behaviors and caries prevalence was evident in the studied population. Similar patterns have been described in pediatric populations, where dental caries remains highly prevalent and is associated with plaque accumulation, the age at initiation of toothbrushing, and inadequate oral hygiene practices.[14,28]

Plaque index and associated factors

In the present study, higher plaque accumulation was observed among girls. Although sex-related differences in plaque accumulation have been inconsistently reported in the literature, this finding may reflect variations in oral hygiene practices, caregiver supervision, and family-related contextual factors within the study population. From a clinical perspective, these differences could also be influenced by behavioral patterns and household dynamics that shape daily oral hygiene routines during childhood.

Public health implications

In agreement with the findings of the present study, Borrell and Talih[29] demonstrated the usefulness of equity-sensitive indicators, such as the symmetrized Theil index, to quantify disparities in dental caries among children and adolescents in the United States. Their work highlights that socioeconomic inequalities constitute a measurable and persistent determinant of oral health, even in high-income countries. This evidence supports the importance of monitoring health inequalities as a basis for guiding targeted public health interventions.

Similarly, Almerich-Torres et al.[30] reported that dental caries in schoolchildren was not significantly associated with body mass index when analyses were stratified by social class, suggesting that socioeconomic conditions may play a more relevant role than nutritional status alone. This finding is consistent with the results of the present study, in which dental caries prevalence was higher among children from economically disadvantaged families, reinforcing the need for integrated, equity-focused public health strategies.

Overall, this study highlights the need for targeted oral health interventions, particularly in settings with limited resources. The importance of improving education, access to care, and preventive strategies is consistent with evidence from multicenter and population-based studies conducted in low- and middle-income countries.[24-26] To be effective, future programs should be designed with careful consideration of the sociodemographic context in which children live.

Limitations and future directions

This study presents certain limitations that should be acknowledged. First, the use of a non-probabilistic sampling strategy restricts the external validity of the findings and limits their generalizability to other populations. Additionally, the relative socioeconomic homogeneity of the study sample may have reduced the ability to detect statistically significant associations between sociodemographic variables and caries outcomes. The reliance on self-reported information from caregivers also introduces the possibility of information bias.

Future research should prioritize longitudinal study designs to better assess caries progression over time and to establish temporal relationships between sociodemographic factors and oral health outcomes. Moreover, the inclusion of additional variables—such as dietary patterns, fluoride exposure, access to preventive dental services, and community-level determinants—would contribute to a more

comprehensive understanding of caries risk and inform the development of targeted public health interventions.

CONCLUSIONS

This study confirmed a high prevalence of dental caries among children aged 5–12 years in Victoria, Cabañas, with a higher burden observed among girls and children from lower-income families. Although no statistically significant associations were identified between maternal education or family income and caries prevalence, the observed trends suggest that socioeconomic vulnerability may still influence oral health outcomes in this population. In addition, a clinically relevant association was identified between poor oral hygiene, as measured by the plaque index, and dental caries occurrence, particularly among girls, despite the absence of statistically significant correlations.

From a public health perspective, these findings underscore the importance of implementing early, age- and sex-specific preventive strategies. The results highlight the need for culturally adapted interventions targeting vulnerable sociodemographic groups, with particular emphasis on oral hygiene education and community-based prevention programs.

The study's limitations include the use of a non-probabilistic sample and the relative sociodemographic homogeneity of the population, which may have limited the detection of statistically significant associations. Consequently, future research should employ longitudinal designs and include more diverse populations to better elucidate the relationships between socioeconomic conditions, oral hygiene practices, and caries progression.

In light of these findings, expanding access to preventive oral health services through school-based programs, mobile dental units, and public health campaigns is essential to reduce caries risk in underserved communities. Policymakers should prioritize the integration of oral health into primary healthcare and educational settings. Furthermore, longitudinal research exploring genetic, environmental, and behavioral determinants is needed to strengthen the evidence base for the development of sustainable, equitable, and effective oral health interventions.

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CONFLICTS OF INTEREST

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Retrospective analysis of guided access technique in calcified canals using cone beam tomography and digital scanning

Bruna de Athayde Casadei[1], Alexandre Sigrist De Martin[1], Rina A Pelerine[1], Wayne Martins Nascimento[1], Daniel Guimarães Pedro Rocha[2], Carlos E Fontana[3], Vinicius de Carvalho Machado[1], Carolina Pessoa Stringheta[1], Ana G Limoeiro[4], Michel Klymus[4], Thais Machado de Carvalho Coutinho[5], Marilia Fagury Videira Marceliano-Alves[5,6,7], Karoline de Oliveira Vieira Clemente[8], Carlos E da Silveira Bueno[1]

1. *Instituto de Pesquisa São Leopoldo Mandic, Faculdade de Odontologia São Leopoldo Mandic, Campinas, SP, Brazil.*
2. *Pontificia Universidade Católica de Campinas, Departamento de Endodontia, Campinas, São Paulo, Brazil*
3. *Pontificia Universidade Católica de Campinas, Programa de Pós-Graduação em Ciências da Saúde,Campinas, São Paulo, Brazil*
4. *Universidade de São Paulo, Faculdade de Odontologia de Bauru, Departamento de Dentística, Endodontia e Materiais Dentários, Bauru, SP, Brazil*
5. *Universidade Iguaçu, Programa de Pós-Graduação em Odontologia, Nova Iguaçu, Rio de Janeiro, Brasil*
6. *Centro Universitário Maurício de Nassau (UNINASSAU), Rio de Janeiro, Brasil*
7. *Department of Dental Research Cell, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pune 411018, India*
8. *Universidade UNILAGOS, Araruama, Brazil*

ABSTRACT

The endodontic treatment of teeth presenting with calcified pulp is associated with significant technical difficulties. ***Aim:*** *To evaluate the success rate of the guided access technique for root canals using cone beam computed tomography (CBCT) between 2019 and 2021.* ***Material and Method:*** *The study selected cases of anterior and posterior teeth with severe pulp calcification, totaling 100 teeth including 59 incisors, 2 canines, 8 premolars, and 31 molars. CBCT scans were performed for all patients, and a virtual burr path was planned on a computer screen to guide the drill to the beginning of the root canal. The virtual drill path was delineated based on axial and cross-sectional tomographic data, and a template was fabricated using Computer Numerical Control (CNC) technology. Success was determined by reaching the canal lumen without deviation from the original path.* ***Results:*** *The success rate of the guided access technique was 99.1%, with calcification diagnosed in 48.7% of cases. Clinical history revealed deep restoration and necrosis in 34.8% of cases, and trauma in 34.8%. In 65.2% of cases, an attempt was made before referral for guided access.* ***Conclusion:*** *The guided access technique demonstrated high success rates, particularly when appropriately indicated.* ***Clinical relevance:*** *Guided access technique provides a reliable option for managing calcified root canals, thereby improving treatment outcomes.*

Keywords: *guided endodontics - cone beam tomography - image guided surgery*

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Corresponding Author:
Ana Grasiela Limoeiro,
grasielalimoeiro@gmail.com

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Análise retrospectiva da técnica de acesso guiado em canais calcificados utilizando tomografia de feixe cônico e escaneamento digital

RESUMO

O tratamento endodôntico de dentes que apresentam polpa calcificada está associado a dificuldades técnicas significativas ***Objetivo:*** *Avaliar a taxa de sucesso da técnica de acesso guiado para canais radiculares usando tomografia computadorizada de feixe cônico (TCCB) entre 2019 e 2021.* ***Material e Método:*** *O estudo selecionou casos de dentes anteriores e posteriores com calcificação pulpar grave, totalizando 100 dentes, incluindo 59 incisivos, 2 caninos, 8 pré-molares e 31 molares. As varreduras de CBCT foram realizadas para todos os pacientes, e um caminho de broca virtual foi planejado em uma tela de computador para guiar a broca até o início do canal radicular. O caminho da broca virtual foi delineado com base em dados tomográficos axiais e transversais, e um molde foi fabricado usando a tecnologia de Controle Numérico Computadorizado (CNC). O sucesso foi determinado ao atingir o lúmen do canal sem desvio do caminho original.* ***Resultados:*** *A taxa de sucesso da técnica de acesso guiado foi de 99,1%, com calcificação diagnosticada em 48,7% dos casos. A história clínica revelou restauração profunda e necrose em 34,8% dos casos, e trauma em 34,8%. Em 65,2% dos casos, uma tentativa foi feita antes do encaminhamento para acesso guiado.* ***Conclusão:*** *A técnica de acesso guiado demonstrou altas taxas de sucesso, particularmente quando bem indicada. Relevância clínica: A técnica de acesso guiado fornece aos clínicos uma opção confiável para o gerenciamento de canais radiculares calcificados, melhorando assim os resultados do tratamento.*

Palavras-chave: *endodontia guiada em 3D - tomografia de feixe cônico - cirurgia guiada por imagem.*

INTRODUCTION

Endodontic treatment of teeth with calcified pulp poses significant technical challenges[1]. The obliteration of the pulp space, often leading to tertiary dentin formation due to caries progression[2] or in deep restorations[3], complicates access to root canals. In addition, trauma-induced obliteration can further exacerbate the situation, making it difficult to perform endodontic procedures[4] such as lateral dislocation, intrusion or avulsion[5-7]. While preventive root canal treatment is not recommended as a routine protocol for teeth with pulp obliteration[8], approximately 10% of cases may develop periapical changes necessitating endodontic intervention after 15 years[9].

Accessing and navigating calcified canals is technically complex, and errors such as deviation from the original path or instrument breakage are common, even with surgical microscopy[10]. Radiographic guidance alone may lead to errors, highlighting the need for advanced techniques[11,12].

The combination of CBCT and digital scanning offers a promising solution for establishing a guide to negotiate calcified root canals[13]. Case reports have demonstrated positive outcomes using this approach in both anterior and posterior teeth[14-17]. The aim of this study was to evaluate the efficacy of the guided access technique in negotiating calcified root canals using CBCT and digital scanning.

MATERIALS AND METHOD

Human teeth were used after securing approval from the Ethics Committee - Instituto de Pesquisas São Leopoldo Mandic (number: 396931200005374). This was a retrospective study that analyzed data from 115 clinical cases conducted between January 2019 and March 2021. To analyze the success rate of the procedure, the initial CBCT image of each case (iCAT; Imaging Sciences International, Hatfield, PA; FOV 5 × 5 cm; voxel size 0.125 mm) and the final radiograph obtained after endodontic treatment were evaluated. Success was defined as the clinician's ability to reach the canal lumen, thereby overcoming pulp calcification.

The professionals who performed the treatments were sent a questionnaire, created using the Google Forms® application (GoogleCorp, Version 2018, Mountain View, California, USA), with the following questions: "How long ago did you graduate?"; "Are you a specialist in endodontics?"; "Did you attempt to access the canal before using a guided endodontic approach?"; "If you attempted access, did you do so with or without an operating microscope?"; "What is the patient's gender?"; "How was the diagnosis of calcification made?"; "Did you successfully access the canal lumen?"

The sample size was calculated using the Epi Info™ software (Centers for Disease control and prevention, available from: https://www.cdc.gov/epiinfo/por/pt_index.html). The sample size of 115 participants enabled the success of the technique to be estimated with a sampling error of 1.7% and a confidence level of 95%. Descriptive and exploratory data analysis was performed using absolute and relative frequencies. Then, the prevalence of clinical history of teeth with pulp calcification, the forms of calcification diagnosis, and the success of the guided access technique were estimated with their respective 95% confidence intervals. All analyses were performed using Program R with a significance level of 5%.

The inclusion criterion for case selection was anterior or posterior tooth with severe pulp calcification. The teeth analyzed were 59 incisors, 2 canines, 8 premolars and 31 molars in which the dentist was unable to reach the lumen of the dental canal, and therefore referred them to Scanning Radiology to make the prototype template. Teeth with metal restorations that could cause imaging artifacts were excluded. A CBCT scan (I-CAT Imaging Sciences International, Inc., Hatfield, PA, USA) was performed on all patients. Based on software designed for implant dentistry (SIM Implant, Sirona Dental Systems, Leuven, Belgium), a virtual burr path with a diameter of 1.3 mm was planned on the computer screen from an incisal reference to the beginning of the radiographically visible root canal. The burr path was designed to reach the first visible part of the root canal using special alignment procedures. After adjusting the angulation of the virtual burr path, it was possible to avoid involvement of the incisal edge and still reach the root canal lumen. A virtual washer was superimposed on the virtual burr path for drill guidance. The axial and cross-sectional tomographic data were used to determine the location of the root canal. Based on this, the virtual drill path was constructed. The teeth were scanned on the surface (R700 Desktop - 3 Shape, Copenhagen, Denmark). The virtual surface models were merged with the CBCT volume,

creating a combined image with a 3D volume of the tooth including the virtual burr path. The template including the metal washer was fabricated using Computer Numerical Control (CNC) technology as SICAT Optiguide (SICAT, Bonn, Germany) based on the combination image. This technology provides higher accuracy. To achieve better stability, the adjacent teeth were included in the template. The drill axis was angled so that the extended drill tip reached the radiographically visible lumen of the root canal (Fig. 1).

After planning the drilling position, a virtual model was designed using the model designer of the Simplant software (Dentsply Sirona). A guide sleeve (outer diameter 3.0 mm, inner diameter 1.4 mm and length 8 mm) was fitted to the drill using a software tool and virtually added to the planning library before the model was created. Fixation washers were also created to stabilize the guide and prevent the drill from deviating from its trajectory created using tomographic planning. The virtual model was exported as an STL file and sent to a 3D printer (Objet Eden 260 V with FullCure 720; Stratasys Ltd, Minneapolis, MN). The washer was incorporated into the printed model to guide the drill during cavity preparation. The dentist followed the protocol for attaching the template and drilling with the drill for access. The success of the guided access technique was assessed by the ability to reach the canal lumen without deviation from the original path.

Statistical analysis

Descriptive and exploratory data analysis was carried out using absolute and relative frequencies. Prevalence rates were then estimated for the clinical histories of teeth with pulp calcification, the ways in which calcification was diagnosed and the success of the guided access technique, with the respective 95% confidence intervals. All the analyses were carried out using the R program, with a significance level of 5%.

RESULTS

The success of the guided access technique consists of reaching the lumen of the canal that the dentist was unable to reach during the first treatment, with or without magnification. Of the 115 cases analyzed, 99.1% were successful, with calcification diagnosed in 48.7% of cases (Table 1). Of the 115 cases of guided access technique for calcified canals analyzed, 70.4% were performed by professionals with more than 10 years' training, of whom 98.3% were endodontists (Fig. 2).

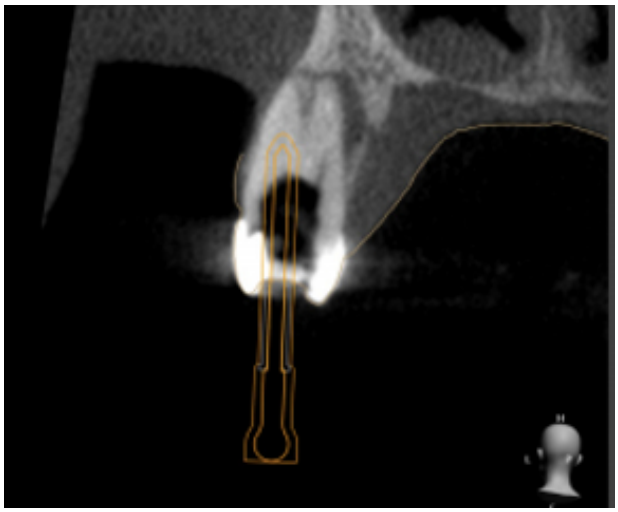

Fig. 1: CBCT with virtual simulation of the drill reaching the lumen of the canal.

Regarding the characteristics of the cases, 80.9% of the patients were female. Deep restoration and necrosis were observed in 34.8% of cases, while trauma was present in 34.8%. Most cases (65.2%) had attempted access before referral for guided access. It should also be noted that in 40.0% of cases, the specialists diagnosed calcification because they had unsuccessfully tried to access it with a microscope, and in one case (0.9%) the specialist unsuccessfully tried to access it with a microscope and cone beam tomography. In 12.2 % of the cases, the diagnosis was made because the dentist unsuccessfully attempted to access the canal without a microscope. Table 2 shows the prevalence with the respective 95% confidence intervals.

The one case of failure occurred in a tooth 21 (central incisor) where the template resulted in a deviation from the original canal course. This case of failure was performed by a professional with more than 10 years' training (Fig. 3).

DISCUSSION

Based on an analysis of 115 clinical cases across various tooth groups, the guided access technique demonstrated high accuracy in both anterior and posterior regions where pulp obliteration occurred due to calcification. This technique involves the integration of tomographic images, virtual planning, and scanning to create a guide that directs the entry of the low-rotation drill.

Table 1. Characteristics of clinical cases in which the guided access technique was used for calcified canals.

Variable	Category	Frequency (%)
Patient gender	Female	80.9
	Male	19.1
Tooth history	Deep restoration and necrosis	34.8
	Old trauma (more than one year)	32.2
	Caries with pulp contamination	8.7
	Complaint of darkening and the patient does not remember trauma in the region.	7.8
	Parafunctional habit	4.3
	Recent trauma (less than one year)	2.6
	Dentinogenesis imperfecta	1.7
	Referred with previous opening	1.7
	Periodontal pocket with pulp necrosis and periradicular abscess	0.9
	Deep caries, prosthetic purpose	0.9
	Symptomatic tooth. Patient does not remember history of trauma	0.9
	Orthodontic history	0.9
	Periapical lesion and symptomatology	0.9
	Tooth crown breakage	0.9
	Previous unsuccessful treatments and persistent pain	0.9
The way the calcification was diagnosed	Unsuccessfully attempted access using a microscope	40.0
	Initial radiographic exam	30.4
	Unsuccessfully attempted to access the canal without a microscope	12.2
	Another practitioner attempted unsuccessful access with microscope	7.8
	Other practitioners attempted unsuccessful access	4.4
	Deviation when attempting fiber abutment removal	2.6
	Tomography	1.7
	Unsuccessfully attempted access using microscopy and cone beam tomography	0.9
Success of the procedure	No	0.9
	Yes	99.1

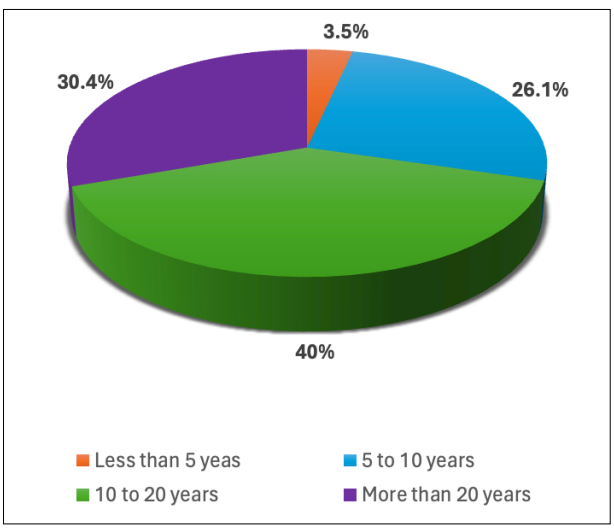

Fig. 2: Distribution of dental surgeons in the cases who underwent the procedure according to years' training.

The tooth structure loss caused by the drill is similar to the space created by a fiberglass post. In simulated teeth with calcification, conventional treatment resulted in greater structural loss compared to guided access localization[18-19]. Additionally, the conventional approach may lead to deviations and dislocations in various directions, thereby increasing the risk of accidents[20].

Advancements in technology, including CBCT, digital planning and 3D printing, have facilitated the introduction of guided endodontics. This study critically evaluated the clinical potential of the guided access technique and outlined the operative steps for safe implementation in treating complex endodontic cases. Primary indications for guided endodontics include accessing calcified root canals, navigating

Table 2. Prevalence and confidence intervals of clinical history in teeth with pulp calcification, form of calcification diagnosis and success of guided access technique procedure

Variable	Category	Prevalence (%)	Confidence intervals 95%
Clinical history of teeth	Deep restoration and necrosis	34.8	26.1% - 43.5%
	Trauma	34.8	26.1% - 43.5%
	Caries with pulp contamination	8.7	3.5% - 13.8%
The way the calcification was diagnosed	Attempted access without a microscope	16.5	9.7% - 23.3%
	Attempted access without a microscope without success	48.7	39.6% - 57.8%
	Initial radiographic exam	30.4	22.0% - 38.8%
Attempted access before guided access	Yes	65.2	56.5% - 73.9%
Success of the technique	Yes	99.1	97.4% - 100.0%

challenging anatomical areas, removing fiberglass posts, and managing teeth with developmental anomalies[21]. Guided endodontics has proven to be a precise, effective, safe, clinically applicable strategy, integrating technological resources and digital planning into endodontic practice[22].

Most of the dentists who participated in this study were specialists with over a decade's experience. They often attempted to gain access to the canal lumen using a surgical microscope before resorting to template fabrication at a radiology center. These findings underscore the significance of the technique in cases where traditional methods may have failed due to limitations in experience or technology. Guided endodontics enables safe access to the canal with reduced procedural time and minimal loss of hard tissue, regardless of the practitioner's expertise level[23].

Limitations of the guided technique include the need for direct, straight access to the canal lumen, as the drill is not curved. Accessing posterior molars can pose challenges, depending on factors such as mouth opening and the risk of microcrack formation due to drill overheating[12,17]. Furthermore, the time and cost involved in the process, including tomographic examination, scanning, case planning, and template fabrication, should be considered1[2]. While cavity preparation for guided access and guided surgery offers high accuracy and success rates, further research with larger patient cohorts is warranted to draw definitive conclusions[24]. Previous studies[25-28] indicate that guided endodontics offers advantages in terms of safety, speed and accessibility for less experienced practitioners. However, this technique also has limitations and the potential for iatrogenesis[29]. These limitations include inadequate fixation of the template to the bone, and inaccuracies in manual mesh fusion, which can lead to root perforations[18]. In addition, static guidance is limited to straight roots or segments of curved roots, requiring longer planning time and higher radiation exposure due to mandatory CBCT scans. Furthermore, the additional costs for CBCT and templates contribute to increased patient costs. Furthermore, the study did not differentiate between single-rooted and multi-rooted teeth, which could affect the results[30].

In conclusion, based on the sample analyzed in this study, the guided technique emerges as a reliable, accurate tool when appropriately indicated. Nonetheless, ongoing research and prospective clinical studies are essential to further substantiate the evidence supporting guided endodontic practice.

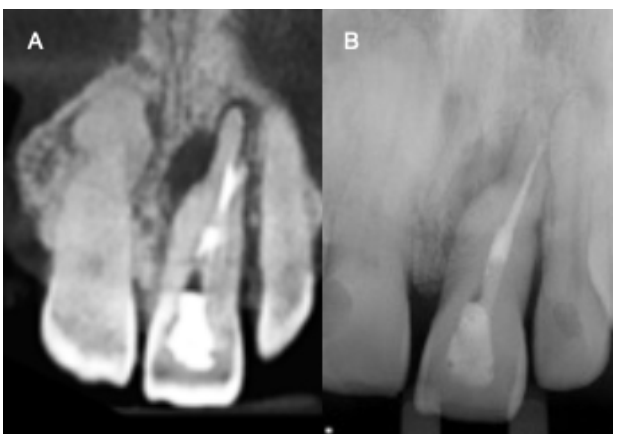

Fig. 3: Failure case: A) CBCT image and B) radiograph, note the deviation from the original canal course.

CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship and/or publication of this article.

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Comparative study of root canal mechanical preparation with two systems with different kinematics. Ex vivo micro-CT study

Li Ting Lee, María E Santillán, Eugenia Miklaszewski, María L Cabirta, Liliana G Sierra, Pablo A Rodríguez

Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Endodoncia, Buenos Aires, Argentina

ABSTRACT

Oval and ribbed root canals present a major challenge in endodontic treatment due to their complex anatomy, which fosters the accumulation of debris and bacteria. Mechanical preparation systems have been developed to improve shaping efficiency and canal centricity in these anatomically complex canals. ***Aim:*** *To compare, using micro-computed tomography, pulp space volume increase, untouched canal surface area, and apical transportation in oval root canals prepared with WaveOne Gold (WOG) or XP-endo Shaper (XPS).* ***Materials and Method:*** *30 extracted single-rooted upper second premolars with oval root canals were randomly divided into two experimental groups and instrumented with WOG (n: 15) or XPS (n: 15). All specimens were scanned with micro-CT (SkyScan 1272; Bruker-microCT) for comparison before and after instrumentation using the following acquisition parameters: 19µm voxel, 30 kV, 800 mA, 0.6° rotation step, 360° rotation and 1 mm thick aluminum filter. Initial and final volumes, surface areas and centricities were analyzed to calculate pulp space volume increase, untouched surface area and transportation. Data were analyzed using paired Student's t-tests, Wilcoxon signed ranks or Mann-Whitney U tests, as appropriate ($p < 0.05$ significant).* ***Results:*** *There was no significant difference between WOG and XPS in pulp space volume increase ($p=0.26$) or untouched surface area ($p>0.99$). However, there were significant differences in centricity on the X and Y axes ($p<0.05$), while on the Z axis, the differences were not significant ($p=0.05$).* ***Conclusion:*** *WOG and XPS had similar shaping effectiveness. All files were able to clean and shape moderately curved canals with minimal apical transportation.*

Keywords: *x ray micro-CT - mechanical preparation - transport - shaping ability - root canal preparation*

Estudio comparativo de preparación mecánica con dos sistemas de diferentes cinemáticas. Evaluación ex vivo mediante micro-CT.

RESUMEN

Los conductos radiculares ovalados y con irregularidades son un desafío importante en el tratamiento endodóntico debido a su anatomía compleja, que favorece la acumulación de detritus y bacterias. Con el objetivo de mejorar la eficiencia del modelado y el centrado del conducto, se han desarrollado distintos sistemas de preparación mecánica para abordar estos conductos anatómicamente complejos. ***Objetivo:*** *comparar mediante micro-CT el incremento de volumen del espacio pulpar, superficie no tocada y transporte apical producido en conductos ovalados tratados con: WOG o XPS.* ***Materiales y métodos:*** *fueron utilizados 30 segundos premolares superiores unirradiculares ovalados, divididos aleatoriamente en dos grupos experimentales instrumentados con: WOG (n:15) y XPS (n:15). Todas las muestras fueron escaneados con micro-CT inicial y post-instrumentación (SkyScan 1272; Bruker-microCT) utilizando parámetros de adquisición: vóxel 19µm, 30 kV, 800 mA, paso de rotación de 0,6°, rotación de 360° y filtro de aluminio de 1 mm de espesor, para ser comparadas entre sí. Se analizaron los volúmenes, superficies y centricidades iniciales y finales para calcular el incremento de volumen del espacio pulpar, superficie no tocadas y transporte. Los datos se analizaron mediante las pruebas t-Student apareada, de los rangos con signos de Wilcoxon o U de Mann-Whitney, según lo que correspondía ($p< 0,05$ significativo).* ***Resultados:*** *No hubo diferencias significativas entre WOG y XPS en el incremento de volumen del espacio pulpar ($p=0,26$) y superficie no tocada ($p>0,99$). Sin embargo, sí hubo diferencias significativas en la diferencia de centricidad en los ejes X e Y ($p<0,05$). En el eje Z, las diferencias no llegaron a ser significativas ($p=0,05$).* ***Conclusión:*** *WOG y XPS tuvieron efectividad de modelado similar. Todas las limas utilizadas fueron capaces de limpiar y dar forma a conductos moderadamente curvados con un mínimo transporte apical.*

Palabras clave: *micro-CT - preparación mecanizada - transporte - desgaste - preparación del conducto radicular*

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Corresponding Author:
Lee, LiTing
lee.ting@odontologia.uba.ar

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INTRODUCTION

The purpose of endodontics is to cure or prevent infectious pathologies of the periapical tissues. During the mechanical preparation of the root canal, no instrument can reach all the multiple irregularities of the internal anatomy, and there may remain untouched areas that harbor organic tissue debris and bacteria. Thus, the chemical action of irrigant solutions is essential to reach an adequate level of conformation and disinfection, facilitating a good three-dimensional seal[1,2]. This is especially important in the preparation of root canals that are not circular in section[3].

Oval and ribbed canals present a challenge in endodontics because their complex anatomy fosters the accumulation of detritus and bacteria. The prevalence of this kind of anatomy is one third of teeth in their apical portion, being more frequent in incisors and lower second premolars[4].

In search of a more centered conformation, with minimum deviations from the main axis of the canal and greater respect for the internal anatomy, the metal alloys used in the systems may be subjected to any of several thermal treatments, and can also present different kinematics such as rotation, oscillation, reciprocating movement, and other adaptive forms[1,2,5,6].

Single file systems, which considerably reduce treatment time, subject each instrument to a high level of cyclic and torsional fatigue. Reciprocating systems have a longer service life than continuous rotation systems[7].

Among the continuous rotation systems is the XP-endo Shaper (XPS) (FKG Dentaire, La Chaux-de-Fond, Switzerland). Its serpentine dynamics and MaxWire alloy body give it superelasticity and shape memory, enabling it to adapt to the three-dimensionality of the canal due to variations caused by temperature changes, thereby improving cleaning and shaping during instrumentation[2,5,8].

Reciprocating kinematics provide an alternative technique to continuous rotation, based on the technique of manual instrumentation of balanced forces, which generates less anatomical distortion and reduces the risk of instrument fracture due to cyclic fatigue[2]. It consists of an oscillatory movement in which the instrument rotates and counter-rotates with different degrees of rotation[7]. This system includes the Wave-One Gold (WOG) (Dentsply Maillefer, Ballaigues, Switzerland), a single file system with surface heat treatment on its gold metal alloy. It has an off-centered parallelogram cross-section with two 85° cutting edges. According to the manufacturer's instructions, it is used with pecking and planing movements[5,8].

X-ray microtomography (Micro-CT) is one of the most accurate methods for the investigation of root canal morphology, being non-invasive, non-destructive and reproducible[8]. It has been used in several studies to compare instrumented vs. non-instrumented areas of different mechanical preparation systems, often on oval internal anatomy[2-4,6,9-11].

The aim of the current study was to compare, by means of X-ray microtomography, the volume increase, uninstrumented surface area, and apical transport produced in oval root canals treated with the WOG or XPS systems.

MATERIALS AND METHOD

Thirty extracted single-rooted second upper premolars were selected according to the following inclusion criteria: single oval canal (type I classification according to Vertucci), complete apexogenesis, and curvature angles between 5° and 15° (mild grade according to Schneider). To corroborate this, a radiovisiograph (Kodak RVG 6100, Kodak) was used and two projections were made for each specimen: buccolingual and mesiodistal.

The specimens were standardized with an axial cut 19 mm in length from the anatomical apex. The selected teeth were stored in a 50% glycerin – 50% alcohol solution, following the methodology used in the Endodontics Department of the School of Dentistry of the University of Buenos Aires.

After standardization of the specimens, an initial microtomographic X-ray scan (SkyScan-1272; Bruker, USA) was performed using the following acquisition parameters: 19μm voxel (resolution), 30kV (current), 800mA (voltage), 0.6° rotation step, 360° rotation around the vertical axis and 1mm thick aluminum filter. The acquired projections were reconstructed using NRecon software (Ruker, Kontich, Belgium). The specimens were randomly divided into two experimental groups to be instrumented with two different systems: group WOG (n=15) and group XPS (n=15).

Instrumentation was performed according to the protocol used in Endodontics Department of the

School of Dentistry of the University of Buenos Aires, and according to the manufacturer's indications for each system.

Subsequently, the samples were scanned again with the same initial parameters. The images were analyzed individually and in a geometric overlay of the pre- and post-instrumentation images with the visualization software Data Viewer v.1.5.1, (Bruker, Kontich, Belgium). Morphometric analysis of the images was performed using the CTan v.1 .14.4 software (Bruker, Kontich, Belgium) analyzing, for the whole canal: 1) Initial dentin volume (VID, mm^3); 2) Final dentin volume (VFD, mm^3); 3) Initial dentin surface (SID, mm^2); 4) Final dentin surface (SFD, mm^2); 5) X, Y, Z centroid pre-instrumentation (mm) and 6) X, Y, Z centroid post-instrumentation (mm). The following variables were calculated:

Endodontic space volume increases according to Gambril et al.[12]

$$\textit{Volumetric increase \%} = \frac{\textit{Final volume} \times 100}{\textit{Initial volume}}$$

The surface area of the uninstrumented endodontic space according to Gambril et al.[12]

$$\textit{Uninstrumented surface area \%} = \frac{\textit{Uninstrumented surface area} \times 100}{\textit{Final surface area}}$$

Transportation was measured by means of the difference in position between the pre- and post-instrumentation centroids on each axis (X, Y, Z) according to Morales et al.[13] Negative numbers represented deviation in the mesial direction, while positive numbers represented deviation in the distal direction.

STATISTICAL ANALYSIS

The data sets were described by the following measures: minimum (Min), maximum (Max), mean, standard deviation (SD), median, first quartile (Q_1) and third quartile (Q_3). To compare two paired data sets, paired Student's *t*-test was used when the normality condition was met; otherwise, Wilcoxon signed-rank test was used. To compare two grouped data sets, the nonparametric Mann-Whitney U test was used. The parametric pooled *t*-Student test was not used because the conditions of normality or homoscedasticity were not met. The assumptions of normality and homoscedasticity were analyzed using the Shapiro Wilk test with modifications, and the F test, respectively. In all inference tests, differences were considered significant when the p-value was less than 0.05 ($p<0.05$). The InfoStat v. 2020 program[14] was used.

RESULTS

With both systems, endodontic space volume increased significantly after instrumentation (Table 1). With the WaveOne Gold system (WOG), the median (Q1/Q3) pre-and post-instrumentation volumes (mm^3) were 7.68 (2.60/8.26) and 8.84 (7.79/9.70), respectively (Wilcoxon signed-rank: $Z=-3.72$; $p<0.05$); and with the XpShaper system (XPS), they were 5.50 (4.68/13.39) and 12.63 (7.87/17.75), respectively (ranges with Wilcoxon signed-rank: $Z=-3.41$; $p<0.05$).

The results for pulp space surface area were analogous to those found for volume. With both systems, the surface areas increased significantly after instrumentation (Table 2). With the WOG, the median (Q_1/Q_3) surfaces (mm^2) pre- and post-instrumentation were 50.63 (41.77/61.32) and 61.30 (60.84/62.50), respectively (Wilcoxon signed-rank: $Z=-3.59$; $p<0.05$); with XPS, they were 73.17 (51.68/78.77) and 92.37 (75.20/98.68), respectively (ranges with Wilcoxon signed-rank: $Z=-3.41$; $p<0.05$).

After the analysis of volume and surface area between instances, calculations were made to compare the percentage increase in pulp space volume and surface area after instrumentation between the two systems.

Regarding the percentage increase in pulp space volume, there was no statistically significant difference (Mann-Whitney U: $W=286.00$; $p=0.26$) (Table 3) between the two experimental groups: the median (Q_1/Q_3) was 33.49% for WOG (15.66/150.00), and 43.02% for XPS (31.81/50.69). For the untouched surface after instrumentation (Fig. 1), there was no statistically significant difference (Mann-Whitney U: $W=255.00$; $p>0.99$) (Table 4) between the two experimental groups: the median (Q_1/Q_3) was 81.45% for WOG (68.86/92.23), and 79.72% for XPS (77.18/80.13).

Table 1. Volume of the pulp space according to the system used, before (Pre) and after (Post) instrumentation.

System	Instance	Volume (mm^3)								p*
		n	Mean	SD	Min	Max	Median	Q_1	Q_3	
WOG	Pre	15	6.10	2.92	1.47	8.80	7.68	2.60	8.26	<0.05
	Post	15	9.04	1.79	6.50	12.31	8.84	7.79	9.70	
XPS	Pre	15	8.71	4.80	4.36	15.61	5.50	4.68	13.39	<0.05
	Post	15	13.03	5.47	6.57	20.35	12.63	7.87	17.75	

**Wilcoxon signed-rank test*

Table 2. Pulp space surface according to the system used, before (Pre) and after (Post) instrumentation.

System	Instance	Surface (mm^2)								p*
		n	Mean	SD	Min	Max	Median	Q_1	Q_3	
WOG	Pre	15	52.55	19.05	25.44	85.46	50.63	41.77	61.32	<0.05
	Post	15	69.30	25.77	46.05	124.08	61.30	60.84	62.50	
XPS	Pre	15	65.12	19.82	34.59	87.47	73.17	51.68	78.77	<0.05
	Post	15	84.12	23.23	44.72	109.63	92.37	75.20	98.68	

**Wilcoxon signed-rank test*

Table 3. Percentage increase in pulp volume after instrumentation, according to the system used.

System	Increase in volume (%)								p*
	n	Mean	SD	Min	Max	Median	Q_1	Q_3	
WOG	15	104.77	139.78	7.43	429.93	33.49	15.66	150.00	0.26
XPS	15	63.44	50.96	30.36	169.87	43.02	31.81	50.69	

**Mann-Whitney U test*

Centricity and Transportation

The centricities of the X, Y and Z axes were compared before (pre) and after (post) instrumentation in order to determine whether there is transportation in the canal. For the X axis, no statistically significant difference was found in the WOG group: the medians

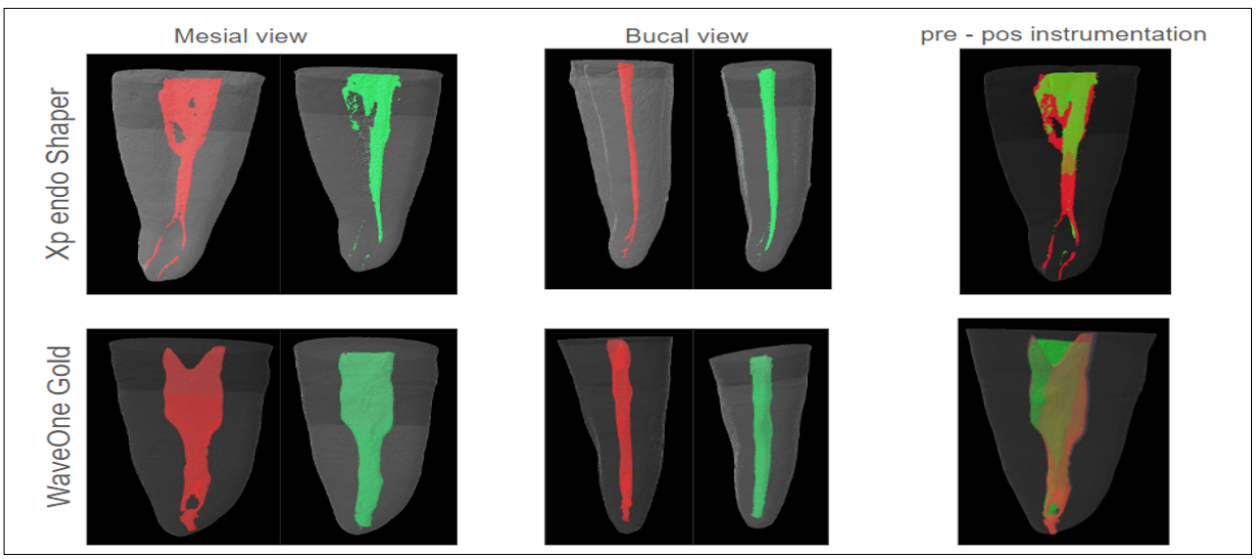

Fig 1: Microcomputed tomography images in mesial view, buccal view and overlay taken before (red) and after (green) the use of rotary and reciprocating instruments reveal areas that were not prepared (red).

Table 4. Percentage of untouched surface, according to the system used.

System	Untouched surface (%)								p*
	n	Mean	SD	Min	Max	Median	Q_1	Q_3	
WOG	15	77.69	20.11	41.40	100.79	81.45	68.86	92.23	>0.99
XPS	15	78.00	13.36	56.00	97.30	79.72	77.18	80.13	

**Mann-Whitney U test*

(Q_1/Q_3) pre- and post-instrumentation were 8.43 (7.64/13.35) and 8.86 (5.77/12.40), respectively (paired t-Student: T=1.69; p=0.11). However, in the XPS group transportation before and after instrumentation differed significantly (Table 5), with medians (Q_1/Q_3) 5.70 (5.16/7.81) and 8.10 (6.46/12.97), respectively (Wilcoxon signed-rank: Z=-3.42; p<0.05).

As in the X-axis, for the Y-axis, no statistically significant difference was found in the WOG group: the medians (Q_1/Q_3) pre- and post-instrumentation (mm) were 8.40 (7.63/12.09) and 9.81 (5.92/12.91),

Table 5. X-axis centricity according to the system used, before (Pre) and after (Post) instrumentation.

System	Instance	X-axis (mm)								p
		n	Mean	SD	Min	Max	Median	Q_1	Q_3	
WOG	Pre	15	9.56	3.14	5.45	13.99	8.43	7.64	13.35	0.11*
	Post	15	8.75	3.69	3.87	12.78	8.86	5.77	12.40	
XPS	Pre	15	6.25	1.40	4.45	8.02	5.70	5.16	7.81	<0.05 **
	Post	15	9.38	3.11	6.26	13.07	8.10	6.46	12.97	

* t-Student paired
***Wilcoxon signed-rank test*

respectively (paired t-Student: T=-0.26; p=0.80). However, there was a statistically significant deviation for the XPS group after instrumentation (Table 6), where the medians (Q_1/Q_3) pre- and post-instrumentation (mm) were 5.51 (5.29/6.96) and 8.30 (6.31/13.66), respectively (Wilcoxon-signed rank: Z=-3.41; p<0.05).

For the Z axis, the results were inverse compared to axes X and Y: in the WOG group, statistically

Table 6. Y-axis centricity according to the system used, before (Pre) and after (Post) instrumentation.

System	Instance	Y-axis (mm)								p
		n	Mean	SD	Min	Max	Median	Q_1	Q_3	
WOG	Pre	15	9.08	2.82	4.87	13.10	8.40	7.63	12.09	0.80*
	Post	15	9.26	3.99	3.66	13.44	9.81	5.92	12.91	
XPS	Pre	15	6.06	1.02	4.91	7.66	5.51	5.29	6.96	<0.05**
	Post	15	9.67	3.51	6.11	13.99	8.30	6.31	13.66	

* t-Student paired
***Wilcoxon signed-rank test*

significant differences in centricity (mm) were found: the medians (Q_1/Q_3) pre- and post-instrumentation were 10.47 (4.77/15.03) and 8.80 (5.87/11.66), respectively (Wilcoxon signed-rank: Z=2.81; p<0.05). However, for the XPS group there was no significant difference before and after instrumentation (Table 7), where the medians (Q_1/Q_3) were 7.46 (5.65/16.87) and 6.84 (5.94/17.64), respectively (ranges with Wilcoxon signed-rank: Z=-1.25; p=0.21).

Table 7. Centricity of the Z axis according to the system used, before (Pre) and after (Post) instrumentation.

System	Instance	Z-axis (mm)								p*
		n	Mean	SD	Min	Max	Median	Q_1	Q_3	
WOG	Pre	15	10.38	5.50	4.28	17.13	10.47	4.77	15.03	<0.05
	Post	15	8.78	3.57	4.19	13.36	8.80	5.87	11.66	
XPS	Pre	15	10.41	5.72	4.60	17.53	7.46	5.65	16.87	0.21
	Post	15	10.69	5.88	5.26	17.84	6.84	5.94	17.64	

**Wilcoxon signed-rank test*

Following the centricity comparisons before and after instrumentation of each system, we compared the centricity differences (transportation, mm) between the systems on each axis. On the X and Y axes, significant differences were found: the difference in centricity (mm) was greater with XPS than with WOG. On the X axis, the median (Q_1/Q_3) was -1.03 (-1.78/0.32) for WOG and 3.55 for XPS (1.17/5.05) (Mann-Whitney U: W=372.00; p<0.05; Table 8). On the Y-axis, the median (Q1/Q3) was 0.26 for WOG (-2.17/1.84) and 3.29 for XPS (0.94/6.11) (Mann-Whitney U: W=345.00; p<0.05; Table 8). On the Z-axis, the differences were not significant: the median (Q_1/Q_3) was -2.49 for WOG (-3.47/1.10) and 0.01 (-.039/1.06) for XPS (Mann-Whitney U: W=309.00; p=0.05; Table 8).

Table 8. Difference in centricity of the X, Y, Z axes post- and pre-instrumentation, according to the system used.

Axis	System n	Difference (mm)								p*
		Mean	SD	Min	Max	Median	Q_1	Q_3		
X	WOG	15	-0.81	2.03	-3.87	2.65	-1.03	-1.78	0.32	<0.05
	XPS	15	3.13	1.97	0.66	5.35	3.55	1.17	5.05	
Y	WOG	15	0.18	2.83	-3.87	4.74	0.26	-2.17	1.84	<0.05
	XPS	15	3.61	2.64	0.80	7.03	3.29	0.94	6.11	
Z	WOG	15	-1.60	2.27	-3.88	1.73	-2.49	-3.47	1.10	0.05
	XPS	15	0.28	0.79	-0.61	1.34	0.01	-0.39	1.06	

**Mann-Whitney U test*

DISCUSSION

This study evaluated canal preparation and transportation in extracted single-rooted upper premolar canals using different instruments. Despite the limitations for sample standardization, the use of extracted teeth remains the best option, since resin-fabricated teeth have a critical limitation in terms of the difference in hardness between dentin and resin[15]. To minimize the variables and achieve better standardization, the specimens were standardized with cuts from the apical limit up to 19mm towards apical, and randomly distributed, resulting in two groups without differences. However, previous "narrowing" of the canals was not taken into account, and this could have influenced the result, since the mostly calcified canals could have shown increased volume. For future studies, it would be interesting to homogenize the sample by standardizing the volume previously. Ane Poly et al.[16], compared the same systems as in our study, and found better centering of XPS with respect to WOG but greater wear with the former. The differences in the results could be due to the fact that the authors measured the volumes of each canal prior to instrumentation by means of a fast X-ray scan.

In the current study, all specimens were handled by the same operator. In contrast, a study by Hofmann analyzing the centering ability of reciprocating instruments and using plastic plugs with curved root canals observed that significant differences between

operators contributed more to canal transport than did the file system itself[15].

Despite differences in cross-sectional design and kinematics that affect the shaping ability of NiTi preparation systems[17-20], we agree with Mamede-Neto I et al., Saberi E et al.[21] and Versiani et al.[17] that there were no significant statistical differences in wear and transportation between rotating and reciprocating systems.

Wu et al.[22] argued that apical canal transportation of less than 0.3 mm would have minimal impact on prognosis. Therefore, the quality of the images evaluated is crucial for the accuracy of the results. Although CBCT is known to produce less detailed images than micro-CT[23-26], CBCT has been used as an evaluation method in recent studies[27-29].

Recent studies with the same purpose and using micro-CT as a 3D evaluation method presented voxel sizes ranging from 20 to 22.8μm. As root canal anatomy gradually changes along the z-axis, a voxel size of 34μm was shown to provide acceptable image quality, while smaller voxel sizes provide greater accuracy for evaluating root canal preparation. The present study therefore used micro-CT as the 3D evaluation method, choosing a voxel size of 18μm with the aim of obtaining better image quality. However, Zanesco et al.[30] showed that the digital subtraction radiographic technique was reliable and there was no statistical difference with micro-CT images in apical transportation analysis.

CONCLUSION

WOG and XPS showed similar shaping effectiveness. All instruments were able to clean and shape moderately curved canals with minimal apical transportation. These results emphasize the relevance of adequate irrigation, since mechanical preparation alone does not ensure complete cleaning of the root canal system.

CONFLICT INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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An ex vivo comparison of bond strength measured by the push-out test between AH Plus and Endosequence BC sealer Hiflow

Thayane da Costa Pinheiro[1], Carlos E da Silveira Bueno[1], Alexandre Sigrist De Martin[1], Carolina Pessoa Stringheta[1], Carlos E Fontana[2], Rina A Pelegrine[1], Ana G Limoeiro[3], Wayne M Nascimento[1], Marilia Fagury Videira Marceliano-Alves[5,6,7], Thais Machado de Carvalho Coutinho[5], Cesar A Perini Rosas[8], Daniel G Pedro Rocha[4]

1. *Faculdade São Leopoldo Mandic, Instituto de Pesquisa São Leopoldo Mandic, Departamento de Endodontia, Campinas, SP, Brasil.*
2. *Pontifícia Universidade Católica de Campinas, Centro de Ciências da Vida, Programa de Pós-Graduação em Ciências da Saúde, Campinas, SP, Brasil.*
3. *Universidade de São Paulo, Faculdade de Odontologia de Bauru, Departamento de Dentística, Endodontia e Materiais Dentários, Bauru, SP, Brazil.*
4. *Pontificia Universidade Católica de Campinas, Departamento de Endodontia, Campinas, São Paulo, Brazil*
5. *Universidade Iguaçu, Programa de Pós-Graduação em Odontologia, Nova Iguaçu, Brasil.*
6. *Centro Universitário Maurício de Nassau, Rio de Janeiro, Brasil.*
7. *Department of Dental Research Cell, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pune 411018, India.*
8. *Universidade Estadual do Norte do Paraná, Campus de Jacarezinho, Paraná, Brasil*

ABSTRACT

Endodontic obturation is fundamental to treatment success, with the bond strength of sealer cements to dentin being a crucial parameter for evaluating their effectiveness. ***Aim:*** *To evaluate the bond strength between root canal dentin walls and the obturation materials AH Plus and Endosequence BC Sealer HiFlow using the push-out test.* ***Material and Method:*** *Twenty single-rooted mandibular premolars were divided into two groups (n=10) according to the sealer used: Group AH (AH Plus Sealer) and Group HF (Endosequence BC Sealer HiFlow). The canals were instrumented with Wave One Gold Large, using 20 mL of 2.5% sodium hypochlorite as irrigation solution. The teeth were cut into 1 mm thick slices in the cervical and middle third regions to perform the push-out test. The failure mode was observed under a stereomicroscope at 40x magnification. The results were subjected to Tukey's test and ANOVA with a 5% significance level.* ***Results:*** *In the push-out bond strength test, there was no statistically significant difference between AH Plus and Endosequence BC Sealer HiFlow cements (p=0.1952). Similarly, the failure modes showed no significant difference between the cements, in both the cervical and middle thirds (p=0.5703).* ***Conclusion:*** *AH Plus and Endosequence BC Sealer HiFlow demonstrated similar behavior in terms of bond strength and failure mode in root canal dentin walls.*

Keywords: *dentine - bond strength - root canal sealers*

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Corresponding Author:
Ana Grasiela Limoeiro
grasielalimoeiro@gmail.com

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Comparação ex vivo da resistência de união do teste de push-out entre os cimentos AH Plus e o Endosequence BC Hiflow

RESUMO

A obturação endodôntica é fundamental para o sucesso do tratamento, sendo a resistência de adesão dos cimentos seladores à dentina um parâmetro crucial para avaliar sua eficácia. ***Objetivo:*** *Avaliar a resistência de adesão das paredes de dentina do canal radicular e a obturação com AH Plus e Endosequence BC Sealer HiFlow pelo teste de push-out.* ***Materiais e Método:*** *Vinte pré-molares mandibulares de raiz única foram divididos em dois grupos (n=10) de acordo com o cimento utilizado: Grupo AH (Cimento AH Plus) e Grupo HF (Endosequence BC Sealer HiFlow). Os canais foram instrumentados com Wave One Gold Large, utilizando um total de 20 mL de hipoclorito de sódio a 2,5% como solução irrigante. Os dentes foram cortados em fatias de 1 mm de espessura nas regiões do terço cervical e médio para realizar o teste de push-out. O modo de falha foi observado sob um estereomicroscópio com aumento de 40x. Os resultados foram submetidos ao teste de Tukey e ANOVA com nível de significância de 5%.* ***Resultados:*** *No teste de resistência de adesão (push-out), não houve diferença estatisticamente significativa entre os cimentos AH Plus e Endosequence BC Sealer HiFlow (p=0.1952). Similarmente, os modos de falha não apresentaram diferença significativa entre os cimentos, tanto no terço cervical quanto no médio (p=0.5703).* ***Conclusão:*** *Os cimentos AH Plus e Endosequence BC Sealer HiFlow demonstraram um comportamento semelhante em termos de resistência de adesão e modo de falha nas paredes de dentina do canal radicular.*

Palavras-chave: *dentina - resistência de adesão - selante de canal radicular*

INTRODUCTION

Endodontic obturation is an essential step to seal the root canal system and prevent future bacterial contamination or recontamination[1]. Endodontic sealers can interact with dentin both physically and chemically. Physical interaction occurs when the material penetrates the dentinal tubules, creating mechanical retention, while chemical interaction is characterized by the formation of tags along the cement-dentin interface[2]. In addition to bond strength to dentin, some obturation materials show a biological response at the material-dentin interface that provides support, improving the sealing quality[3]. Leakage and filling material bond strength have been used to evaluate the effectiveness of root canal fillings[4]. The push-out test is commonly used to assess the bond strength between cement and canal walls[5].

AH Plus (AHP - Dentsply Maillefer, Tulsa, OK, USA) is an epoxy resin-based endodontic cement that is considered the gold standard due to its good adaptation and bond strength compared to other materials[6]. It also has a long-term sealing function, short setting time, and better flowability than other cements[7].

Bioceramic sealers are promising materials to improve the filling quality and thus the long-term success of endodontic treatment[8]. Endosequence BC Sealer HiFlow (HF -Brasseler, Savannah, GA) is a bioceramic sealer in premixed syringe form that has sufficient biological properties to be safely used as a root canal filling material[9]. Its composition is similar to that of the standard Endosequence BC Sealer (BC), with a base of calcium silicate, monobasic calcium phosphate, calcium hydroxide, zirconium oxide and thickeners[10], and it has antimicrobial activity, high pH, hydrophilicity, and diffusion of calcium hydroxide[11].

The aim of this study was to compare AHP and HF by performing an *in vitro* evaluation of bond strength between the endodontic sealers and root canal dentin walls using the push-out test. The null hypothesis is that the tested groups have equivalent results in terms of the analyzed parameters.

MATERIALS AND METHOD

Specimen selection and preparation:

This study was approved by the Human Research Ethics Committee of the São Leopoldo Dental College Research Center (CAAE: 38675120.0.0000.5374). Twenty freshly extracted permanent single-rooted human mandibular premolars were donated by patients who had an indication for extraction for various reasons, with each patient having signed an informed consent form. The inclusion criteria were fully formed single-rooted teeth with a single root canal, without fractures, calcifications, cracks or previous endodontic treatment. The teeth were divided into 2 groups (n=10 per group) according to the sample calculation (G Power 3.1.9.4, Franz Faul, College of Kiel, Germany) with a type "a" error of 0.05 and a type "b" error of 0.80. Specimens were preserved in 0.1% thymol solution.

Periapical radiographs were acquired in the mesiodistal and buccolingual directions to confirm the presence of a single straight circular canal using the New Ida digital sensor (Dabi-Atlante Ltda, Ribeirão Preto, Brazil). These specimens were also examined with an optical surgical microscope (Alliance, São Carlos, Brazil) at 16× magnification to confirm the absence of cracks, fractures and external apical resorption on the entire surface of each root. The teeth were cleaned with periodontal curettes (Duflex- SS White Artigos Dental Ltda., Rio de Janeiro, Brazil), ultrasound (Schuster Equipamentos Odontologicos, Santa Maria, Brazil) and water. After cleaning, the coronal part of each tooth was cut with a double-sided diamond disc (KG Sorensen, Barueri, Brazil) to standardize root length to 15 mm.

The teeth were placed in a container of impression compound with condensation silicone (Panasil, Indaiatuba, SP, Brazil) for stabilization. Apices were sealed with wax (Wilson Polidental Ind. e Com. Ltda., Cotia, Brazil). Subsequently, all endodontic treatments were performed by a specialist in endodontics.

Odontometry was determined visually with a size 10 K-file (Maillefer Corp, Ballaigues, Switzerland) inserted into each canal to verify that it was in the apical foramen based on a periapical radiograph. The working length (WL) was determined at the apical foramen (0,0). Then the canals were preflared in the cervical third with the Orifice Shaper (17/.08) rotary file (MK Life, Porto Alegre, Brazil). Instrumentation was performed with 15/.02, 20/.02, 25/.02 Flexofile hand files and Wave One Gold Large (45/.05) reciprocal file (Dentsply Sirona, Ballaigues, Switzerland) connected to the VDW Silver endodontic motor (VDW, Munich, Germany) in WaveOne ALL mode.

During root canal cleaning and shaping, a 5-mL irrigation syringe (Ultradent Products, Indaiatuba, Brazil) was used to irrigate with 2.5% NaOCl (Asfer Ind. Química Ltda. São Caetano do Sul, Brazil), for a total of 20 mL of solution per tooth. A final ultrasonic irrigation was performed with an Irrisonic E1 insert (Helse Ultrasonic, Santa Rosa de Viterbo, Brazil), stirring the rinse solution for 20 seconds with 17% ethylenediaminetetraacetic acid (EDTA) (Biodinamica, Ibiporã, Brazil) in 3 cycles (1 mL per cycle); 3 additional cycles (2 mL per cycle) for 20 seconds with 2.5% NaOCl, and finally with 5 mL of distilled water. Then, the canals were dried using a capillary tip (Ultradent Products, Indaiatuba, Brazil) and absorbent paper points (MK Life, Porto Alegre, Brazil).

Subsequently, the samples were randomly (www.randon.org) divided into 2 groups of 10 samples:

- Group AHP: AH Plus Sealer
- Group HF: Endosequence BC Sealer HiFlow

The obturation was performed using a VDW single cone (Dentsply Ind. e Com. Ltda, São Paulo, Brazil) compatible with the canal diameter. After root canal filling, the teeth were kept in an incubator at 100% humidity and 37ºC for 1 week to allow the sealers to set completely. All the bond strength tests were conducted by a blinded operator who did not know the experimental groups.

Bond strength evaluation

The roots were cut into 1mm thick slices in a slicer (Isomet 1000 Precision Saw, Buehler, Canada) with a disk (EXTEC Dia. wafering blade) and placed in containers of distilled water to hydrate, separated by group and number of specimens. They were then subjected to the push-out test using the universal testing machine EMIC, model DL2000, to assess the bond strength between the filling material and the dentin. The specimens were placed individually on the metal surface of the machine. Shortly thereafter, a cylindrical plunger with a diameter of 1 mm for the coronal specimens and 0.6 mm diameter plunger for the middle specimens was inserted, applying pressure in the canal to displace the filling material. The force in Newtons was then converted into tensile strength (in MPa).

Analysis of fault modes

The specimens were evaluated under a stereomicroscope (Stemi 508; Carl Zeiss, Jena, Germany) at 40× magnification to determine the nature of the gap between the dentin walls of the canal and the filling material based on the following scores: 1) adhesive to dentin; 2) adhesive to obturation material; 3) mixed; 4) cohesive in dentin; 5) cohesive in filling material.

Statistical analysis

The results were analyzed using the Biostat 5.3 program and subjected to the Shapiro-Wilk normality test. The sample showed normal behavior, and the parametric ANOVA test was performed with a significance of 5%.

RESULTS

There was no statistically significant difference between the AHP and HF bonding tests in the cervical and middle thirds (p=0.1952), or between the cervical and middle thirds in comparison with the same sealer (p=0.1952) (Table 1).

In terms of failure, there was no statistically significant difference in failure modes between AHP and HF sealers in the cervical and middle thirds (p=0.5703), or between the cervical and middle thirds when comparisons were made with the same sealer (p=0.5703) (Table 2). Types of failure that occurred in this work: adhesive to dentin (Fig. 1A), mixed (Fig. 1B) and cohesive to dentin (Fig. 1C).

Table 1. Arithmetic means, standard deviations, and ANOVA statistical test of bond strength using the push-out test (kgf).

	Coronal	Middle	(p)
AHP	3.37 (1.95)^{A}	4.65 (1.21)^{A}	0.1952
HF	3.79 (2.11)^{A}	4.26 (1.43)^{A}	
(p)	0.1952		

Same capital letters in horizontal and vertical directions: no statistically significant difference.

Table 2. Medians, interquartile deviations, and Kruskal-Wallis statistical test for the failure modes of the sample groups.

	Coronal	Middle	(p)
AHP	3.50 (1.00)^{A}	3.00 (0.00)^{A}	0.5703
HF	3.50 (1.00)^{A}	4.00 (3.00)^{A}	
(p)	0.5703		

Same capital letters in horizontal and vertical directions: no statistically significant difference.

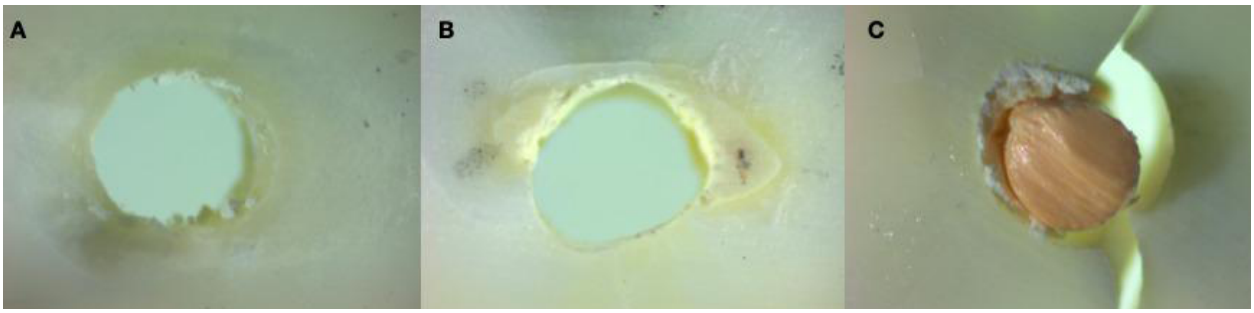

Fig. 1: A) Adhesive failure to dentin, B) Mixed failure and C) Cohesive failure in dentin.

DISCUSSION

In this study, the adhesion between the filling material and the canal walls was evaluated using the push-out test. The AHP and HF sealers showed equivalent results in terms of the parameters analyzed. There was no statistically significant difference in the tests performed, so the null hypothesis was accepted.

The type of movement (rotary or reciprocating) used for canal instrumentation may influence bond strength, as it may create debris that prevents the filling material from bonding to the dentin[12]. In this study, the reciprocating WaveOne Gold instrument was used, and the smear layer was removed from the dentin wall with 17% EDTA. However, Shokouhinejad et al. reported that the presence of a smear layer had no effect on the bond strength of Endosequence BC Sealer and AHP sealers 7 days after root canal filling[13].

The moisture condition of the canal may also affect the bond strength, influence the curing time, and negatively affect the microhardness of the sealer after curing[14]. In the current study, the curing time was 7 days in an oven at 37°C and 100% humidity, and the canals were completely dry, resulting in adequate curing. It may be beneficial to leave the canals slightly moist before filling with bioceramics; however, Nagas et al.[15] found that AHP showed similar results with or without moisture. Srivastava et al.[16] reports that irrigation protocols influenced the push-out bond strength of BioRoot RCS and AHP, while Gibby et al.[17] found that the moisture remaining in the dentinal tubules after canal irrigation could vary greatly even after drying the canals with paper points. As with AHP, such moisture would also reduce monomer conversion, resulting in incomplete polymerization of the resin and lower bond strength to dentin[18].

Bond strength may vary depending on the type of sealer and the root canal filling technique[19]. In contrast to the current study, Hammar et al.[20] reported that the use of AHP with the single cone technique showed a lower bond strength, which could be related to the fact that the high sealer volume reduces the quality of the obturation seal compared to other techniques. However, in another study by Belsare et al. using the lateral condensation technique, AHP showed better bond strength than BC, which was even better when the thermocompaction technique (continuous wave technique) was used[21].

In oval canals, the canal instrumentation and cleaning method and the materials used for obturation can both influence bond strength[3]. Pawar et al.[3] reported that the lowest bond strength was measured in canals instrumented with WaveOne and filled with gutta-percha and AHP. The shear bond strength of dentin to calcium silicate-based sealer was significantly higher than that of AHP. Banphakarn et al.[10] observed residues of calcium silicate sealer adhering to impregnated gutta-percha in most of the tested samples.

In the current study, adhesion failure was mostly cohesive in both groups. This result agrees with Shokouhinejad et al.[13], who investigated AHP and BC sealers. However, in the study by DeLong et al.[22], most failures were mixed when BC was used with the single-cone or continuous wave technique. Al-Hiyasat et al.[19] found that for TotalFill and AHP, the mixed failure mode was the most common, followed by cohesive failure and adhesive failure. This discrepancy in failure modes may be due to the different types of obturation techniques used in the studies.

The limitation of the present study is that it is an *ex vivo* study, which is slightly different from an in vivo study where the tooth is seated in its socket and the periodontal ligament is at body temperature, which may affect sealer properties. Although the bond strength test cannot predict the clinical behavior of

materials, the push-out test is still one of the best measurements of bond strength currently available[23]. Factors such as sample thickness, plunger diameter, and specimen orientation influence the value of the push-out bond strength[24]. All these factors were considered in this study. No other study has yet been conducted to test the bond strength of Endosequence BC Sealer HiFlow, so further research is required on this topic.

CONCLUSION

Based on comprehensive ex vivo evaluation, this study concluded that AH Plus and Endosequence BC Sealer HiFlow had comparable performance. No statistically significant difference was observed in terms of push-out bond strength or failure modes between these two endodontic sealers for either cervical or middle root thirds, thus supporting the null hypothesis. These findings suggest that both materials offer similar bonding capabilities to root canal dentin walls after a seven-day curing period.

CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Histological evaluation of dentin–pulp tissue response after transplantation of stem cells from periosteum and human deciduous teeth and in rats

Tainara B Yamanaka[1], Randro M Rajab[1], Rina A Pelegrine[1], Carlos E da Silveira Bueno[1], Alexandre Sigrist De Martin[1], Carolina Pessoa Stringheta[1], Elizabeth Ferreira Martinez[3], Carlos E Fontana[4], Sérgio L Pinheiro[4], Daniel Guimarães Pedro Rocha[5], Larissa Regina Kuntze dos Santos[1], André A Pelegrine[2]

1 *Faculdade São Leopoldo Mandic, Instituto de Pesquisas São Leopoldo Mandic, Departament of Endodontics, Campinas, SP, Brazil.*
2 *Faculdade São Leopoldo Mandic, Instituto de Pesquisas São Leopoldo Mandic, Departament of Implant Dentistry, Campinas, SP, Brazil.*
3 *Faculdade São Leopoldo Mandic, Instituto de Pesquisas São Leopoldo Mandic, Departament of Oral Pathology and Cell Biology Division, Campinas, SP, Brazil.*
4 *PUC-Campinas, Center of Life Sciences, Postgraduate Program in Health Sciences, Campinas, São Paulo, Brazil.*
5 *PUC-Campinas, Center of Life Sciences, Departament of Endodontics, Campinas, São Paulo, Brazil.*

ABSTRACT

Endodontic treatment offers a high success rate; however, the search for therapeutic alternatives that promote regeneration of the dentin-pulp complex has elicited great interest. ***Aim:*** *The aim of this study was to evaluate, in an in vivo model, the potential for dentin-pulp regeneration by transplantation of stem cells from two sources: pulp of human deciduous teeth and periosteum.* ***Materials and Method:*** *Eighteen mesiobuccal canals of maxillary first molars of immunosuppressed rats were distributed into three groups (n=6): group DT (deciduous teeth pulp), received stem cells from deciduous tooth pulp in hydrogel vehicle; group P (periosteal), received periosteal stem cells in hydrogel vehicle; and group NC (negative control) received only hydrogel. After pulpectomy and canal preparation, the teeth received the respective materials, and the cavities were sealed. After 12 weeks, the animals were euthanized and the specimens underwent histological processing and qualitative evaluation of intracanal fibrous connective tissue, odontoblast-like cells, intracanal mineralized tissue, and inflammatory cell infiltrate.* ***Results:*** *In the NC group, abundant presence of inflammatory cells was observed throughout the canal, in addition to dentin chips and remnants of amorphous substance from pulp tissue. In groups DT and P, in the apical region, new tissue formation suggestive of repair was observed, with deposition of bone-like mineralized tissue and periodontal ligament-like connective tissue containing blood vessels and osteocyte- and fibroblast-like cells.* ***Conclusions:*** *Transplanted stem cells from pulp of human deciduous teeth and periosteum did not show potential for regeneration, but for tissue repair, with formation of periodontal ligament-like and bone-like tissue.*

Keywords: *regeneration - dental pulp - periosteum - rats - histology*

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Corresponding Author:
André Antonio Pelegrine
andre.pelegrine@slmandic.edu.br

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Avaliação histológica da resposta do tecido dentino-pulpar após transplante de células-tronco do periósteo e de dentes decíduos humanos e em ratos

RESUMO

O tratamento endodôntico oferece alta taxa de sucesso; contudo, a busca por alternativas terapêuticas que promovam regeneração do complexo dentino-pulpar tem despertado grande interesse. ***Objetivo:*** *O objetivo deste estudo foi avaliar, em modelo in vivo, o potencial de regeneração dentino-pulpar mediante transplante de células-tronco provenientes de duas fontes: polpa dentária de dentes decíduos e periósteo humanos.* ***Materiais e Método:*** *Dezoito canais mesiovestibulares de primeiros molares superiores de ratos imunossuprimidos foram distribuídos em três grupos (n=6): grupo DT (polpa de dentes decíduos), recebeu células-tronco da polpa de dentes decíduos veiculadas em hidrogel; grupo P (periósteo), recebeu células-tronco do periósteo veiculadas em hidrogel; e grupo NC (controle negativo), recebeu apenas hidrogel. Após pulpectomia e preparo dos canais, os dentes receberam os respectivos materiais e as cavidades foram seladas. Após 12 semanas, os animais foram eutanasiados, e os espécimes submetidos a processamento histológico e avaliação qualitativa de tecido conjuntivo fibroso intracanal, células semelhantes a odontoblastos, tecido mineralizado intracanal e infiltrado celular inflamatório.* ***Resultados:*** *No grupo NC, observou-se presença abundante de células inflamatórias ao longo de todo o canal, além de raspas de dentina e restos de substância amorfa do tecido pulpar. Nos grupos DT e P, na região apical, observou-se neoformação tecidual sugestiva de reparo, com deposição de tecido mineralizado semelhante a osso e de tecido conjuntivo semelhante ao ligamento periodontal, contendo vasos sanguíneos e células semelhantes a osteócitos e fibroblastos.* ***Conclusões:*** *Os transplantes de células-tronco da polpa de dentes decíduos e do periósteo não apresentaram potencial de regeneração, mas de reparo tecidual, com formação de tecido semelhante ao ligamento periodontal e osso.*

Palavras-chave: *regeneração - polpa dentária - periósteo - ratos - histologia*

INTRODUCTION

Dentoalveolar trauma and carious lesions often lead to the development of pulpitis and pulp necrosis, which are commonly treated by pulp removal and replacement with inorganic materials (gutta-percha and endodontic cement). Although current endodontic treatment modalities offer high success rates, altered or necrotic pulp tissue should optimally be removed and replaced with new healthy, vital pulp tissue[1-2].

Tissue engineering is the field of science that seeks to restore function, structure, and physiology in tissues compromised by disease or trauma. It is based on the triad of stem cells, signaling molecules (e.g., growth factors), and a scaffold, the medium which will serve as the structural framework for cells during tissue formation[3-4].

Regeneration has been defined as "the restoration of damaged tissue by tissue similar to the original tissue with concomitant return of biological function"[5]. Therefore, regeneration of the dentin-pulp complex translates into formation of a tissue identical to the damaged native pulp, with the exact same architecture and cellular distribution, composed of fibroblasts, odontoblasts, stem cells, and host defense cells, as well as vascularized and reinnervated[6]. The regenerated tissue must be able to provide sensation, supply nutrition, form new dentin, and mount a response to infection just as the original dentin-pulp complex[4].

Essentially, two pathways to pulp regeneration have been researched: cell recruitment or stem-cell transplantation. In the first model, a scaffold doped with chemotactic signaling molecules is inserted into a previously prepared root canal so that endogenous progenitor cells are recruited into the canal to form new tissue[4]. In the second model, stem cells are isolated, cultured, and added to a scaffold, which is then transplanted directly into the previously prepared root canal[7-8].

Gronthos et al.[9] were the first to isolate dental pulp stem cells (DPSCs): mesenchymal cells with multipotential capacity to differentiate into cells similar to those which make up pulp tissue and therefore act as precursors for tissue regeneration in endodontics. In addition to DPSCs, other mesenchymal stem cells have been identified and characterized, such as stem cells of apical papilla (SCAPs), pulp stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), bone marrow-derived mesenchymal stem cells (BM-MSCs), and cultured autogenous periosteal cells (CAPCs), among others[10-12].

To make stem-cell transplantation for pulp regeneration a viable procedure in everyday dental practice, research has been carried out in animal models, with ectopic, semi-orthotopic, and orthotopic transplants[13]. Clinical research in this line has also advanced, with several reports of outcomes in human patients[8,14-17]. However, these studies have only been able to provide subjective data from imaging modalities and pulp sensitivity testing[8]. Rats approximately 3 months of age were used in this study which have complete root formation and, therefore, minimal relationship between the dentin-pulp complex and the periapical tissue. This fact guided this study with the purpose of evaluating the possible regenerative potential of the dentin-pulp complex in immunosuppressed rats in pulp conditions similar to those of an adult human being [18]. There are already reports in the literature on the regenerative potential of stem cells from human deciduous teeth[19-20]; however, to the best of our knowledge, to date there have been no published reports of research evaluating the potential of periosteal stem cells to regenerate the dentin-pulp complex. As it can be readily accessed throughout the life span via a single superficial tissue layer in the oral cavity, the periosteum is an extremely advantageous source of stem cells[21].

In view of the foregoing and given the vast range of possible combinations of the elements of the tissue engineering triad, regenerative endodontic therapy is a broad field for research seeking to consolidate the much-desired goal of regeneration of the dentin-pulp complex and integrate it into the clinical armamentarium of endodontics practice. The objective of the present study was to evaluate, in an in vivo model, the potential for dentin–pulp complex regeneration after transplantation of stem cells derived from human exfoliated deciduous teeth and periosteum into rat molars with complete root formation.

MATERIALS AND METHOD

Cryopreserved SHEDs and CAPCs, obtained through primary culture, were donated by R-Crio (Campinas, SP, Brazil), as approved by the Brazilian National Research Ethics Committee

(Certificate of Submission for Ethical Approval: 52269321.5.0000.5374; 52217321.7.0000.5375).
Sample size calculation was based on prior work by Pelegrine et al.[22]. One-way ANOVA was performed (G Power 3.1.9.4, Franz Faul, University of Kiel, Germany) with $\alpha = 0.05$, $\beta = 0.80$, and effect size $f = 3.40$. The minimum sample size was calculated as 6 per group.

Animal Selection and Preparation

Once the study protocol had been approved by the Institutional Animal Care and Use Committee (protocol nos. 2021/31 and 2021/32), male Wistar rats (*Rattus norvegicus albinus*; age 12 weeks, weight ~300 g) were selected. All experiments were performed in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and the National Research Council *Guide for the Care and Use of Laboratory Animals*.
Considering the possibility of rejection of human cells, an immunosuppression protocol was started 10 days before the experiment, as described by Lekhooa et al.[23], and continued until euthanasia. The animals were anesthetized by intraperitoneal injection of 10% ketamine hydrochloride 50 mg/mL (Quetamina®; Vetnil, Louveira, SP, Brazil) and 2% xylazine hydrochloride 10 mg/mL (Sedanil®; Vetnil). The animals were placed in dorsal decumbency on a wooden platform. To serve as a mouth gag, orthodontic elastics were wrapped around the incisors of both arches and the other end attached to the platform on which the animal was placed. To keep the cheeks apart, small retractors were made from #08 orthodontic wire (Morelli, Sorocaba, SP, Brazil) folded into a convenient shape.

Study Groups

Eighteen mesiobuccal canals of maxillary first molars from 12 rats were distributed into three groups (n=6 each): in Group DT (deciduous teeth pulp), canals received stem cells from human exfoliated deciduous teeth in hydrogel vehicle; in Group P (periosteal), canals received periosteal stem cells in hydrogel vehicle; and in Group NC (negative control), canals received only hydrogel. For the DT and P groups, the same six animals underwent the transplant procedure, with the maxillary first molars on the right side receiving SHEDs and the maxillary first molars on the left side receiving CAPCs. For the NC group, another six animals were used.

Endodontic Procedures

Access to the pulp chamber was obtained with a No. 1/2 long-shank high-speed round carbide bur (Microdont, São Paulo, Brazil) under refrigeration. After locating the mesiobuccal canal and irrigating the pulp chamber with 2.5% NaOCl (Fórmula e Ação, São Paulo, Brazil), the canal was explored with a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) and the working length established 1 mm short of the apical foramen, with the aid of an electronic apex locator (Propex pixi; Dentsply Sirona, Ballaigues, Switzerland). Instrumentation was performed with Medium (35.06) WaveOne Gold system files (Dentsply Sirona, Ballaigues, Switzerland), driven by an X-Smart Plus engine (Dentsply, Ballaigues, Switzerland) set to the WAVEONE GOLD program. The tooth was instrumented in thirds with small-amplitude in-and-out movements ("pecking motion") until the working length was reached. After every three pecking motions, the canal was irrigated with 1 mL 2.5% NaOCl via a NaviTip 30-gauge needle (Ultradent, South Jordan, UT, EUA) and hypodermic syringe (Injex, São Paulo, Brazil), and apical patency was confirmed with a #10 K-file. After chemomechanical preparation, the canal was irrigated with 1 mL of ethylenediaminetetraacetic acid (EDTA) 17% (Fórmula e Ação, São Paulo, Brazil) for 1 minute and 1 mL of 0.9% sodium chloride (Fórmula e Ação, São Paulo, Brazil). Final aspiration was performed with a Capillary Tip (Ultradent, South Jordan, UT, USA), and the canal was then dried with absorbent paper points (Dentsply Maillefer).

Hydrogel Compounding and Stem Cell Transplantation

The vehicle for stem cell delivery was a 1:1 chitosan/xanthan gum-based hydrogel (1×10^6/mL). The method used to obtain the hydrogel was based on a 1:1 mass ratio of chitosan to xanthan gum[24]. To do so, a 1% w/v chitosan solution (Sigma-Aldrich, Cat. No. 448877, 83% deacetylation) was prepared in a solution composed of 2% v/v lactic acid (Merck, Cat. No. W261106) dissolved in ultrapure water (Milli-Q Direct Q 8/16 System) and homogenized in a mechanical overhead stirrer with a pitched-blade (turbine-type) impeller (Tecnal), at a rotation speed of 1000 rpm. Then, xanthan gum (Sigma, Cat. No. G1253) was dissolved at 1% w/v in ultrapure water (Milli-Q Direct Q 8/16 System) and homogenized in the overhead stirrer at 1000 rpm.

Finally, 100 mL of the chitosan solution was added to 100 mL of the xanthan gum solution with the aid of a peristaltic pump (Tecnal) at a rate of 5 mL/min, temperature of 25°C, under constant stirring at 2000 rpm. After homogenization was complete, stirring at 2000 rpm continued for an additional 5 minutes.

For the *in vivo* experiment, the chitosan/xanthan gum hydrogel was combined with the mesenchymal stem cells. The advanced therapy product was manufactured by mixing, in a 1:1 ratio, sterilized chitosan/xanthan hydrogel with a final concentration of 1.0×10^6 cells/mL resuspended in culture medium (Human Mesenchymal-XF Expansion Medium, Sigma–Aldrich) supplemented with 10% v/v human serum (Sigma–Aldrich, H4522), 1% v/v L-glutamine (Sigma–Aldrich), and 1.1% v/v penicillin/streptomycin (Sigma–Aldrich).

In groups DT and P, approximately 25 µL of the stem cell/hydrogel solution was instilled into the prepared root canal using a 30-gauge NaviTip needle attached to a 1 mL syringe. A Teflon barrier (Isotape®; TDV, Pomerode, SC, Brazil) was then placed and the cavity was sealed with a layer of mineral trioxide aggregate (MTA; Angelus, Londrina, PR, Brazil), followed by a layer of light-curing glass ionomer cement (Ionofast®; Biodynamics, PR, Ibiporã, Brazil). In the NC group, the procedure was similar, but the root canal was filled with hydrogel alone. After 12 weeks, the animals were euthanized by saturation with 2.5% isoflurane (Isoforine®; Cristália, São Paulo, SP, Brazil).

Histological Processing

Specimens obtained from the maxilla were fixed in 10% buffered formalin for 24 hours, decalcified in a 20% formic acid solution for 10 days, and rinsed under running water for 24 hours. They were then dehydrated in a graded ethanol series, cleared in xylene, and embedded in histological paraffin. For histological evaluation, serial longitudinal sections 4 µm thick were obtained, stained with hematoxylin-eosin, and subsequently mounted on glass slides with Permount Mounting Medium (Fisher Scientific, Fair Lawn, NJ, USA).

Images of the slides were captured with a computerized acquisition system (AxioVision Rel. 4.8; Carl Zeiss, Oberkochen, Germany) coupled to a microscope (Axioskop 2 Plus; Carl Zeiss) and evaluated by a single examiner blinded to group allocation.

The following data were collected and qualitatively analyzed: intracanal fibrous connective tissue; morphologically odontoblast-like cells; cementum-like and/or bone-like and/or periodontal ligament-like intracanal mineralized tissue; inflammatory cell infiltrate; and presence of dentin chips.

RESULTS

In samples from the NC group, histological analysis revealed an abundant, predominantly polymorphonuclear inflammatory infiltrate throughout the entire canal, in addition to dentin chips resulting from the instrumentation procedure and remnants of amorphous substance from pulp tissue (Fig. 1).

On histological examination of specimens from groups DT and P, dentin chips, remains of amorphous substance from pulp tissue, and mononuclear inflammatory infiltrate were also observed; however, the latter was less evident. Furthermore,

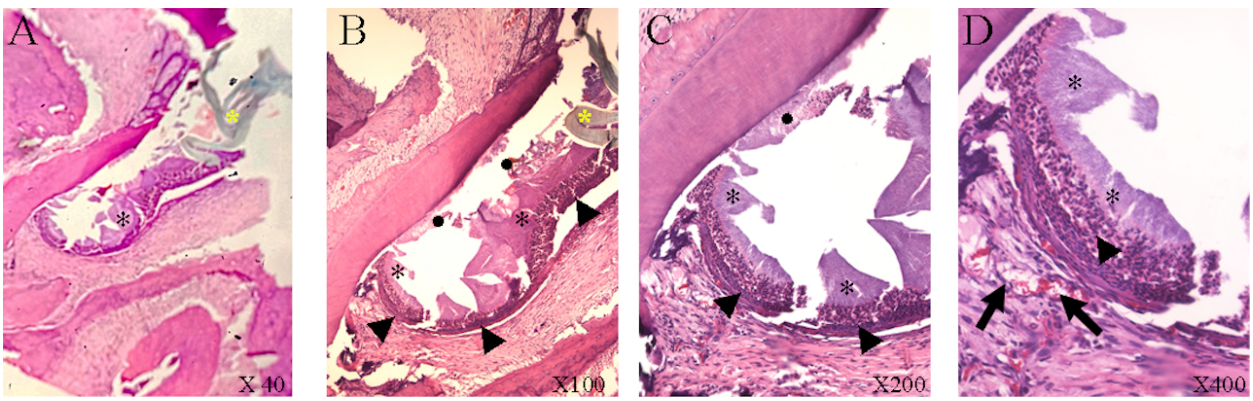

Fig. 1: Representative photomicrograph of longitudinal sections of a mesiobuccal root from a maxillary first molar of a rat from the NC group (H&E; X40, X100, X200, X400 original magnification). Note representative images of dentin chips (*), amorphous substance (•), polymorphonuclear inflammatory cells (►), and blood vessels (↑). The yellow asterisk shows the Teflon barrier.

in the apical region of the root canal (intracanal), new tissue formation suggestive of repair was observed, with deposition of a bone-like basophilic material consistent with mineralized tissue and of a loose, periodontal ligament-like connective tissue, which included the presence of blood vessels and osteocyte- and fibroblast-like cells (Figs. 2, 3).

DISCUSSION

Modern endodontics practice requires harmony between good technique and the best science. Once this balance is achieved, the objective is to ensure health, maintain and/or restore function, and guarantee the longevity of the treated teeth. Regenerative endodontic therapy is becoming tantalizingly closer to daily clinical practice. Advances in research, as well as in our understanding of the mechanisms that guide tissue engineering, have cleared the way towards achieving the much-desired goal of regeneration of the dentin-pulp complex. The present study evaluated the potential of stem cell transplantation to regenerate the dentin-pulp complex, since the cell recruitment strategy commonly results in tissue repair, not regeneration[5]. Stem cells are indispensable in the tissue engineering triad. As noted above, there are several sources of stem cells with potential for regeneration of the dentin-pulp complex: DPSCs[7-9, 25-26], SCAPs[12,27], BM-MSCs[16], umbilical cord mesenchymal stem cells (UCMSCs)[14], and SHEDs[19], among others. In a clinical trial, Xuan et al.[19] evaluated the regenerative potential of DPSCs and observed, in the histological analysis of a single case, complete regeneration of the dentin-pulp complex, concluding that this cell type

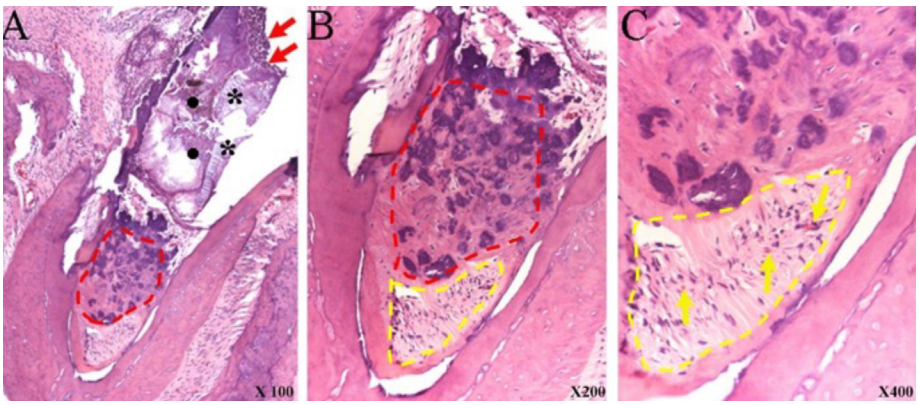

Fig. 2: Representative photomicrograph of longitudinal sections of a mesiobuccal root from a maxillary first molar of a rat from the DT group (H&E; X100, X200, X400 Original magnification). Note the presence of dentin chips (*), amorphous substance (•), polymorphonuclear inflammatory cells (red arrows), blood vessels (yellow arrows). The dotted lines represent bone-like mineralized tissue (red) and periodontal ligament-like loose connective tissue (yellow).

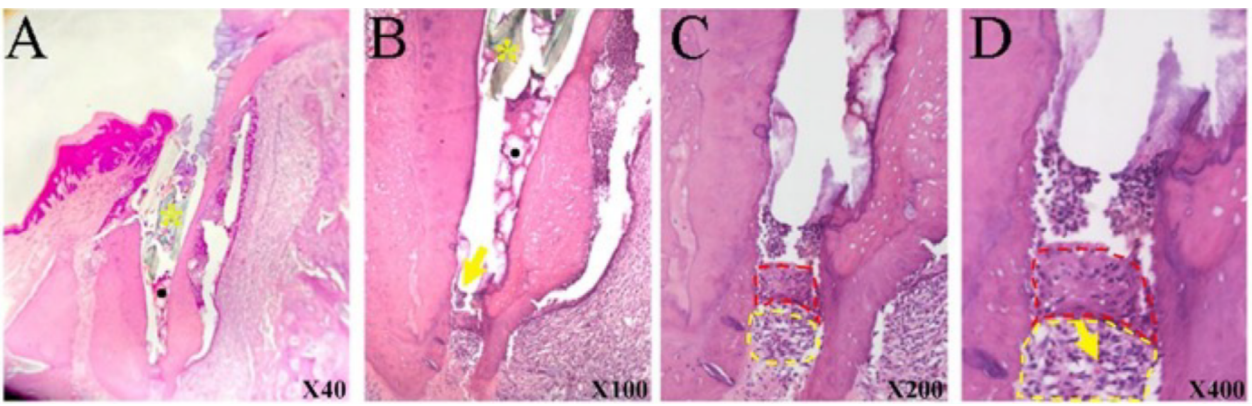

Fig. 3: Representative photomicrograph of longitudinal sections of a mesiobuccal root from a maxillary first molar of a rat from the P group (H&E; X40, X100, X200, X400 original magnification). Note representative images of amorphous substance (•), polymorphonuclear inflammatory cells (red arrow), and blood vessels (yellow arrow). The dotted lines represent bone-like mineralized tissue (red) and periodontal ligament-like loose connective tissue (yellow). (*) Teflon barrier.

was safe and efficacious for this purpose. Conversely, research recently published by our group[20], despite confirming the presence of odontoblast-like cells, found that the transplantation of SHEDs to rat molars resulted in the formation of a cementum-like reparative tissue rather than regeneration. Regarding stem cells of periosteal origin, to the best of our knowledge, there is no research reporting their use in regenerative endodontic therapy, despite their ease of collection[28].

Several groups have advanced research into regenerative therapies by conducting clinical trials with promising results[8,14-16,19]. However, the vast range of possible combinations of the elements of the tissue engineering triad and the lack of standardization in regenerative endodontic therapy protocols mean both *in vitro* and *in vivo* research pursuits are still warranted. Animal studies allow histological examination of newly formed tissues, which is a major limitation of clinical trials. In this context, the use of animal models, especially rat models, is well established in the literature[9,12,27,29]. Nevertheless, the use of orthotopic and heterotopic models is uncommon. A systematic review found only 10 studies using orthotopic models between 2010 and 2017, only one of which was in rats[25].

Rats were selected for the present study because this species combines several desirable characteristics of an animal model, namely: affordable cost, ease of ethical approval, similarity to the human genome, and speed in achieving endpoints of interest[30]. One month of life in a rat is comparable to 3 years of human life; therefore, significant results can be obtained in relatively little time[31]. In this context, we chose to use 12-week-old rats, an age at which complete root formation is already present but degenerative changes are absent[18]. Furthermore, the 12-week period between the experiment and euthanasia is commonly reported in the literature, justifying its adoption in the present study[32-33].

Due to their anatomical, physiological, histological, and biological characteristics, the first molars of rats can be considered miniatures of human molars and, although their small dimensions make cell transplantation difficult, it is not impossible[34]. The mesiobuccal root was chosen to carry out the experiment for its high buccal slope, ease of access, clearer distinction from other canals, and larger volume[35].

No histological similarity was observed between the newly formed tissue seen in the DT and P groups and normal pulp tissue. Contradicting the results of Xuan et al.[19] and corroborating those of Zhu et al.[7] and Santos et al.[20], the present study did not observe any regenerative potential of dental pulp stem cell transplantation but did observe tissue repair potential. Likewise, periosteal stem cell transplantation – to the best of our knowledge, not assessed for this purpose in any previous study – also demonstrated only tissue repair potential rather than any regenerative potential.

Among several factors that may explain these findings, a particularly relevant one is the blood supply of the intracanal region[13]. Adequate blood supply is of great importance to promote tissue regeneration, considering its ability to promote angiogenesis, and ensure the nutrition of stem cells in a timely fashion. The small size of the apical foramen can render this supply difficult, especially in molars[36], hence impairing cell nutrition throughout the entire length of the root canal and potentially explaining a recurring observation in samples from groups DT and P with new tissue formation restricted to the apical region. Huang et al.[37] reiterated the importance of establishing a protocol that would allow satisfactory vascularization, thus enabling cell development up to the cervical third of the tooth. According to the authors, combined transplantation of endothelial cells, administration of growth factors, or apical enlargement – approaches not attempted in the present experiment – could meet this need. Taking together, these factors can explain the pattern of newly formed tissue observed in this trial. In a recent study, Buss et al.[38] evaluated the potential for bone regeneration after filling of critical calvarial defects in rats with xenografts combined with conditioned medium obtained from a stem-cell culture of human dental pulp lineage. Immunohistochemical analysis for markers of angiogenesis (VEGF and anti-CD34) showed that vascularization was stimulated by the conditioned medium. In future research, this could potentially be tested in the intracanal region to evaluate the potential for dentin-pulp complex regeneration.

A key finding in our histological analyses was the abundant presence of acute inflammatory cells throughout the length of the canal in samples from the NC group, whereas this was much less evident in the DT and P groups. This may have been due to the immunomodulatory characteristics of dental

pulp stem cells and periosteal stem cells, which can release anti-inflammatory cytokines such as IL-6 and IL-8 as well as growth factors implicated in angiogenesis, such as VEGF, FGF, and PDGF[39]. This would explain the development of a more favorable microenvironment in the specimens which received stem cell transplants.

Another important finding was the presence of debris (dentin chips and pulp tissue remnants) in all groups. The persistence of debris within the root canal system after chemomechanical preparation is well established in the endodontic literature, since current endodontic instruments are incapable of touching all dentin walls and irrigation/agitation is unable to remove all mineral debris[40-41]. Our results corroborate these well-established findings, indicating that instrumentation was unable to cover all dentin walls and that irrigation, in turn, was unable to remove all the debris generated by instrumentation.

The conception of this study was to outline a protocol for animal model research to transplant stem cells from two different sources into the minute space of the rat root canal system. Future studies could evaluate the regenerative potential of cell transplantation in conjunction with other elements that might enhance its outcomes, e.g., growth factors, other cell types that stimulate angiogenesis/neurogenesis, and apical enlargement.

In an animal model, transplantation of stem cells derived from dental pulp and periosteum resulted in partial new tissue formation in the dentin-pulp complex. Further studies are needed to consolidate a predictable protocol for regeneration of the dentin-pulp complex in mature human teeth.

CONCLUSIONS

Within the limitations of the present study, we conclude that transplanted stem cells obtained from the pulp of deciduous teeth and periosteal stem cells, delivered in a hydrogel scaffold, did not show potential for pulp regeneration, but rather for root canal tissue repair, with formation of intracanal vascularized fibrous tissue and bone-like tissue.

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CONFLICT OF INTERESTS

The authors deny any conflicts of interest related to this study.

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Midpalatal suture expansion: an in vivo histological and immunohistochemical study of the impact of force magnitude

Mónica I Yamauchi[1], Rubén Piña Lugo[2], Luciana M Sánchez[2], Romina C De Lucca[2], Carola B Bozal[2]

1. Sociedad Argentina de Ortodoncia, Ciudad Autónoma de Buenos Aires, Argentina.
2. Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Histología y Embriología, Ciudad Autónoma de Buenos Aires, Argentina.

ABSTRACT

Although there is sufficient clinical evidence regarding the effectiveness of rapid expansion of the middle palate, some of the biological responses to variables such as the magnitude of the expansive force are still unknown. ***Aim*****:** *The aim of this study was to conduct a qualitative and quantitative analysis of the response of midpalatal suture tissues in growing rats exposed to expansive forces of different magnitudes.* ***Materials and Method:*** *Twenty-four 7-week-old male Wistar rats were divided into 3 groups: a control group (C) and two experimental groups to which 60 g or 90 g expansive forces were applied for 7 days (E60 and E90, respectively). Frontal sections of the upper maxillae were stained with haematoxylin-eosin, Masson's trichrome and PAS-Alcian Blue. The expression of runt-related transcription factor 2 (Runx2) and receptor activator of nuclear factor kappa B ligand (RANKL) was evaluated by immunohistochemical staining.* ***Results:*** *The degree of expansion and the biological response of the cells involved in the suture ossification process were evaluated histomorphometrically. ANOVA was used for statistical comparisons. Both the expansive forces applied caused significant increases in maxillary width and suture area. Group E60 showed qualitative changes in the composition of the cartilage extracellular matrix, a significant increase in the percentage of Runx2+ mesenchymal-like cells, and a significant reduction in the number of RANKL+ chondrocytes/mm².* ***Conclusions*****:** *These results show that lower expansive forces would stimulate osteogenesis in a direct manner not associated to the endochondral ossification of suture secondary cartilage, enabling partial elucidation of how bone resorption and formation are regulated during rapid maxillary expansion.*

Keywords: *maxillary expansion - sutures - orthodontic force - animal models - histology*

Expansión de la sutura palatina: Estudio histológico e inmunohistoquímico *in vivo* del impacto de la magnitud de la fuerza

RESUMEN

Existen suficientes pruebas clínicas sobre la eficacia de la expansión rápida del paladar medio; sin embargo, aún se desconocen algunas de las respuestas biológicas a variables como la magnitud de la fuerza expansiva. ***Objetivo*****:** *El objetivo de este estudio fue realizar un análisis cualitativo y cuantitativo de la respuesta de los tejidos de la sutura medio-palatina en ratas en crecimiento expuestas a fuerzas expansivas de diferentes magnitudes.* ***Métodos*****:** *Veinticuatro ratas Wistar macho de siete semanas de edad fueron divididas en tres grupos: un grupo de control (C) y dos grupos experimentales a los que se aplicaron fuerzas expansivas de 60 g o 90 g respectivamente durante siete días (E60 y E90). Se obtuvieron cortes frontales del maxilar superior que fueron teñidos con hematoxilina-eosina (H&E), tricrómico de Masson y PAS-Alcian Blue. También se evaluó la expresión del factor de transcripción Runx2 y del ligando activador del receptor nuclear kappa B (RANKL) mediante inmunohistoquímica.* ***Resultados*****:** *se evaluaron histomorfométricamente el grado de expansión y la respuesta biológica de las células implicadas en el proceso de osificación de la sutura. Se utilizó ANOVA para las comparaciones estadísticas. Ambas fuerzas expansivas aplicadas provocaron aumentos significativos en el ancho maxilar y el área de la sutura. El grupo E60 mostró cambios cualitativos en la composición de la matriz extracelular del cartílago, un aumento significativo en el porcentaje de células tipo-mesenquimales Runx2+ y una reducción significativa en el número de condrocitos RANKL+/mm2.* ***Conclusiones*****:** *estos resultados muestran que las fuerzas expansivas más leves estimularían la osteogénesis de forma directa, sin relación con la osificación endocondral del cartílago secundario de la sutura, lo que permite elucidar parcialmente cómo se regulan la resorción y la formación óseas durante el proceso de la expansión rápida del maxilar.*

Palabras clave: *expansión maxilar – suturas - fuerza ortodóntica - modelos animales - histología*

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Corresponding Author:
Carola B Bozal
carola.bozal@odontologia.uba.ar

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INTRODUCTION

In orthodontics, the usual treatment for narrow upper maxilla is rapid midpalatal expansion (RME), which involves opening the midpalatal suture, with ensuing formation of new bone tissue[1,2]. Although there is sufficient clinical evidence on the effectiveness of this procedure[3,4], it is of particular interest to learn more about the biological response of underlying tissues to the different variables involved, such the magnitude of the expansive force, the time for which the force is applied, the activation protocols, and patient age at the time of implementation. Expansion protocols may use stronger forces for quick expansion or lighter forces for stable expansion.

Various experimental animal models of midpalatal suture expansion have been developed to evaluate the effect of this treatment[5-7]. The present study applied a model of midpalatal suture expansion in rats using a stainless-steel wire spring which generates a calibrated preload expansive force that is equivalent over the entire zone of application.

Histologically, the midpalatal suture in rodents consists of secondary cartilage containing mesenchymal-like cells and osteochondroprogenitor cells[8]. The latter have high proliferative activity and the unique ability to differentiate into chondrocytes or osteoblasts, depending on external biomechanical factors[9]. The midpalatal suture responds to lighter expansive forces by stimulating osteogenic differentiation of this secondary cartilage, generating endochondral ossification[9,10]. Initially, the secondary cartilage present in the suture opens and separates towards the laterals of the palatal bone with active proliferation of chondrocytes. Subsequently, osteoprogenitor cells of the periosteum migrate towards the interior of the suture and then differentiate into osteoblasts[6,10,11].

The endochondral ossification process also involves recruitment of osteoclasts to resorb hypertrophied calcified cartilage, which will subsequently be replaced by new bone. Important in this mechanism are factors that regulate bone resorption and formation such as RANKL and transcription factor Runx2. Hypertrophic chondrocytes express RANKL, thereby regulating precursor recruitment and osteoclast formation[12]. Maxillary suture expansion yields increased expression of the bone remodelling markers such as RANK, RANKL and OPG[13,14]. Runx2 regulates the final differentiation of chondrocytes during endochondral ossification by inducing the passage from chondrocyte in proliferation to hypertrophic chondrocyte, which is necessary for this ossification to proceed normally. This is why Runx2, through activation of the Wnt/β-catenin signalling pathway, is the main transcriptional factor associated with osteoblastic differentiation and endochondral ossification[15,16]. Cartilage extracellular matrix components mediate suture expansion osteogenesis through the Wnt/β-catenin signalling activated Runx2 pathway in osteoblasts[17].

Many of the cell responses in the osteogenesis process triggered by expansive forces, and whether lower-magnitude forces might generate a preferable response pattern, remain unknown to date. Therefore, to better understand the cell responses involved in expansion, as well as the effect of expansive forces of different magnitudes on the response of suture cartilage, the aim of this study was to conduct a qualitative and quantitative analysis of the response of midpalatal suture tissues in growing rats exposed to expansive forces of different magnitudes.

MATERIALS AND METHOD

Experimental units

Twenty-four 7-week-old male Wistar rats (250 to 270g bw) were randomly divided into 3 groups: a control group (C, n=8) and two experimental groups in which rats were equipped with an expansion spring exerting an initial force of either 60 g (E60, n=8) or 90 g (E90, n=8). The rats were housed in galvanized steel cages, with three animals per cage, at 21–24 ºC and 52–56% humidity, under 12h light/dark cycles. The animals had free access to food (standard diet rat-mouse chow, Cooperación) and water.

The experimental protocol was approved by the Ethics Committee of Buenos Aires University's School of Dentistry (Res CICUAL 003/2023) and complied with the ARRIVE guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals[18].

Maxillary expansion protocol

The expansive forces were achieved by means of an orthodontic appliance consisting of a stainless-steel wire spring 30 mm long (Strainght Wire Ortho Organizers, Inc., USA) with an expansion loop

in the centre that determined a 10 mm aperture at its free ends (Fig. 1a-b). Springs made of .014" and .016" stainless-steel wires receive a preload force of approximately 60g (E60) and 90g (E90), respectively. The force was calibrated with an *ad hoc* device consisting of an electronic scale and a dynamometer.

The appliances were installed while the animals were under general anaesthesia by intraperitoneal (IP) injection of ketamine at a dose of 50 mg/kg bw (5 %) and xylazine at a dose of 20 mg/kg bw (2%).

The springs used to generate expansive forces on the midpalatal suture were attached to the palatal surfaces of the upper molars using the direct bonding technique. The procedure for installing the springs involved cleaning the molars manually with pumice powder and a brush, rinsing with water, aspiration, drying with air jet, and acid etching with 37% phosphoric acid on the palatal surfaces of the first, second and third right and left molars of the maxilla. The expander spring was positioned with the loop towards the incisors. Light-curing adhesive (Transbond XT, 3M Unitek) was used with its applicable primer (MIP, 3M Unitek), covering the palatal and occlusal molar surfaces. The animals in group C underwent the same procedures except for installation of the expander spring.

Seven days after installation, the animals were anesthetized by intraperitoneal injection of xylazine (5 mg/kg, König Laboratory) and ketamine (50 mg/kg, Holliday Laboratory) and euthanized by intracardiac injection of 0.2 ml of euthanyl (Brouwer Laboratory). The upper maxillae were resected for further processing.

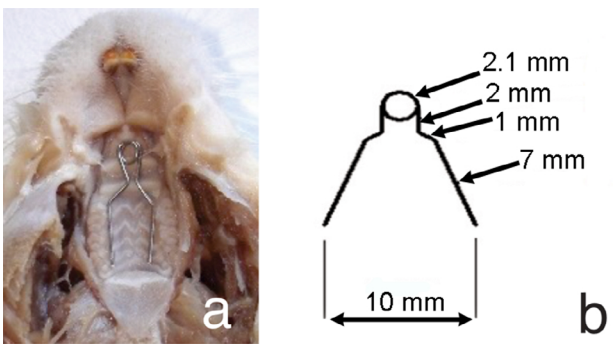

Fig. 1: Methodology: Maxillary expansion
a) Occlusal view of rat upper maxilla with device installed, resected post-euthanasia. b) Diagram showing the attached loop and force direction. The diagram shows the original design with the measurements in millimeters for each section of the spring.

Sample processing

Specimens were fixed in 10% formaldehyde-buffer solution pH 7.4 at 4 ºC and then decalcified in 4M EDTA in 0.4M NaOH (Anedra) pH 7.2 at 4 ºC for 60 days, after which they were processed histologically and embedded in paraffin. Frontal sections 7-8 µm thick were cut at the level of the first upper molars. Histological sections were stained with haematoxylin-eosin (H&E), Masson's trichrome and Periodic Acid-Schiff-Alcian blue stain (PAS-AB). The expression of runt-related transcription factor 2 (Runx2) and of receptor activator of nuclear factor kappa B ligand (RANKL) in the suture tissue was evaluated by immunohistochemical staining.

The sections for immunohistochemical examination were deparaffinized in xylol for 15 min and hydrated in decreasing concentrations of ethyl-alcohol to a final concentration of 70%. For antigen retrieval, the sections were incubated with 0.1% trypsin in tris-maleate buffer, pH 7, at 37 °C for 10 min. The sections were then incubated in 3% hydrogen peroxide in methanol to block endogenous peroxidase. To block nonspecific binding, excess fluid was drained, and the sections were dried and incubated with 1% BSA in PBS 0.01M in a humidity chamber at room temperature for 1 h. The sections were then washed in 0.01M PBS for 5 min, and in normal horse serum (Vector Laboratories, Burlingame, CA, USA) for 10 min. The reaction was detected using a peroxidase-biotin streptavidin system (PK-7800, Vector Laboratories, Burlingame, CA, USA). After that, the sections were incubated in streptavidin peroxidase complex in a humidity chamber at room temperature for 5 min and washed twice in 0.01M PBS at 50 rpm for 5 min each time. The primary antibody was developed with DAB brown stain (DAB substrate kit for peroxidase, SK-4100, Vector Laboratories, Burlingame, CA, USA), and haematoxylin (RANKL) and methyl green (Runx2) counterstain, following the manufacturer's instructions.

RANKL was detected by incubation with primary anti-rat anti-RANKL antibody (AF462, R&D Systems Inc., Minneapolis, MN, USA) at a concentration of 5 µg/mL. RUNX2 was detected by incubation with primary anti-RUNX2 antibody ([EPR14334] Abcam, UK) 1:1000 dilution in PBS 0.01M, in a humidity chamber at 4 °C for 12 h. Negative controls were created by replacing the primary antibody with normal serum (BioGenex

Lab., CA, USA). Bone specimens with proven positive staining by our laboratory were used as positive control.

Histological and histomorphometric evaluation

Photomicrographs of the histological sections were acquired using a light-field microscope (Axioskop 2; Carl Zeiss, Jena, Germany) and a digital camera (Nikon CoolPix 12 Mp, Japan), and analysed histomorphometrically with Image Pro Plus Software®, version 5.1 (Media Cybernetics).

Histological sections cut at the level of the middle roots of the first upper molars were analyzed by light microscopy to define the study area and identify the sites where the measurements were performed (Fig. 2):

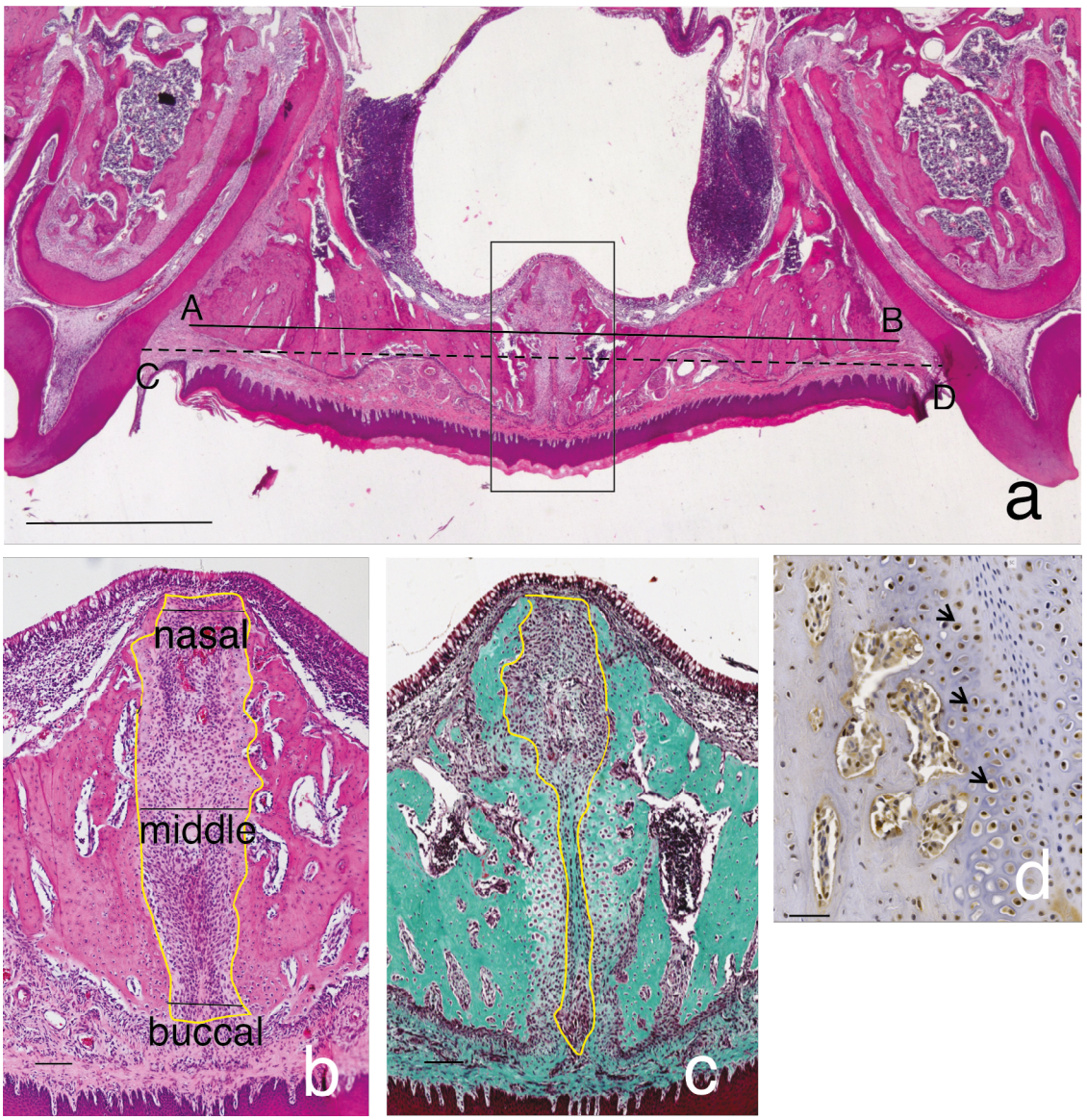

Fig. 2: Methodology: Histomorphometry
a) Photomicrograph of a section at the level of the middle roots of the first upper molars showing right and left molars and the study area of the midpalatal suture (rectangle) observed by light microscopy. H&E, original magnification 10X. Measurements: Maxillary width (µm): continuous line between points A and B, Intermolar width (µm): dashed line between points C and D. Scale 1000 µm. b) Photomicrograph of a section of the midpalatal suture. H&E, original magnification 20X. Measurements: Suture area (µm2): area marked by the yellow perimeter, Suture width (µm): measured at three different levels: nasal, middle and buccal. Scale 100 µm. c) Photomicrograph of a section of the midpalatal suture. Masson's trichrome, original magnification 20X. Measurements: Fibrous area (µm2): area marked by the yellow perimeter. Scale 100 µm. d) Photomicrograph of a section of the midpalatal suture. Runx2 immunostaining contrasted with haematoxylin, original magnification 40X. Chondrocytes with brown stained nuclei were considered positive (arrow). Scale 50 µm.

- Transverse expansion, determined by measuring maxillary and intermolar widths:
  a) Maxillary width (μm): distance between the points determined at the level of the bone crests of the palatal alveolar cortical plates of the right and left first molars (point A to point B) (Fig. 2a)
  b) Intermolar width (μm): distance between the points determined at the level of the cementoenamel junctions on the palatal surfaces of the right and left first molars (point C to point D) (Fig. 2a)
- Suture area (μm^2) (Fig. 2b)
- Suture width (μm) (Fig. 2b) measured at three different levels: nasal, middle and buccal
- Fibrous area (μm^2) (Fig. 2c): fibrous tissue inside the suture area.

Immunohistochemical staining was quantified twice by one examiner with standardized training. The following parameters were determined on immunohistochemically stained sections by direct visualization under a light microscope:

- % Runx2+ C: The percentage of Runx2-positive chondrocytes in the cartilage area was calculated based on the total number of chondrocytes (Runx2-positive plus Runx2-negative chondrocytes). Chondrocytes with brown stained nuclei were considered positive. An average of 60-80 chondrocytes from the total suture cartilage were analysed per sample (Fig. 2d).
- N Runx2+C/mm^2: The number of Runx2-positive chondrocytes per mm^2 in the cartilage area was counted.
- Percentage of Runx2+ (% Runx2+ MC): The percentage of Runx2-positive mesenchymal-like cells in the fibrous area of the suture was calculated based on the total number of mesenchymal-like cells (Runx2-positive plus Runx2-negative mesenchymal-like cells). An average of 120-160 mesenchymal-like cells from the total suture fibrous area were analysed per sample.
- N Runx2+MC/mm^2: The number of Runx2-positive mesenchymal-like cells per mm^2 in the cartilage area was counted.
- N RANKL+C/mm^2: The number of RANKL-positive chondrocytes per mm^2 in the cartilage area was counted.

Statistical analysis

Results were expressed as mean ± standard deviation. The data were analysed statistically by one-way ANOVA (Tukey's multiple comparisons test) to compare groups, using GraphPad Prism Software. Values of $p<0.05$ were considered statistically significant.

RESULTS

Maxillary expansion in groups E60 and E90 were observed in the histological images of sections stained with haematoxylin and eosin (Fig. 3). In the expanded groups, greater intermolar width and a larger suture area were observed in relation to the control group. No obvious inflammatory reaction was found in the area of the midpalatal suture in any of the groups. Figure 4 illustrates with higher magnification the histological and immunohistochemical changes observed after application of the expansive forces. Masson's trichrome stained sections showed that the normal midpalatal suture in group C consisted of a uniform mass of cartilage containing chondrocytes covering the edges of the palatal bone. On the nasal and oral surfaces of the palatal bone, there was a fibrous layer with periosteal cells. After the expansion in groups E60 and E90, the layers of cartilage tissue were forced apart laterally by the mechanical tension force, and the fibrous tissue from the periosteum (with a large number of mesenchymal-like cells) penetrated and migrated into the suture. Cartilage tissue was marked using PAS-Alcian blue staining (PAS-AB). The acid mucin content in the cartilage matrix was high in the central portion of the suture (AB+), especially in group C, while in the periphery of the cartilaginous matrix and the central portion of the suture in groups E60 and E90, there was an increase in PAS+ marking. Midpalatal sutures of animals from groups E60 and E90 showed an increase in Runx2+ mesenchymal-like cells compared to the control, while midpalatal sutures of animals in group E60 clearly showed smaller quantity of RANKL+ chondrocytes compared to animals from groups C and E90.

Transverse measurements showed that in the expansion groups (E60 and E90), both maxillary width and intermolar width increased significantly after suture expansion (Fig. 5a-b). Suture width increased significantly in the central area in group E90 compared to the control (Fig. 5c-e). Suture

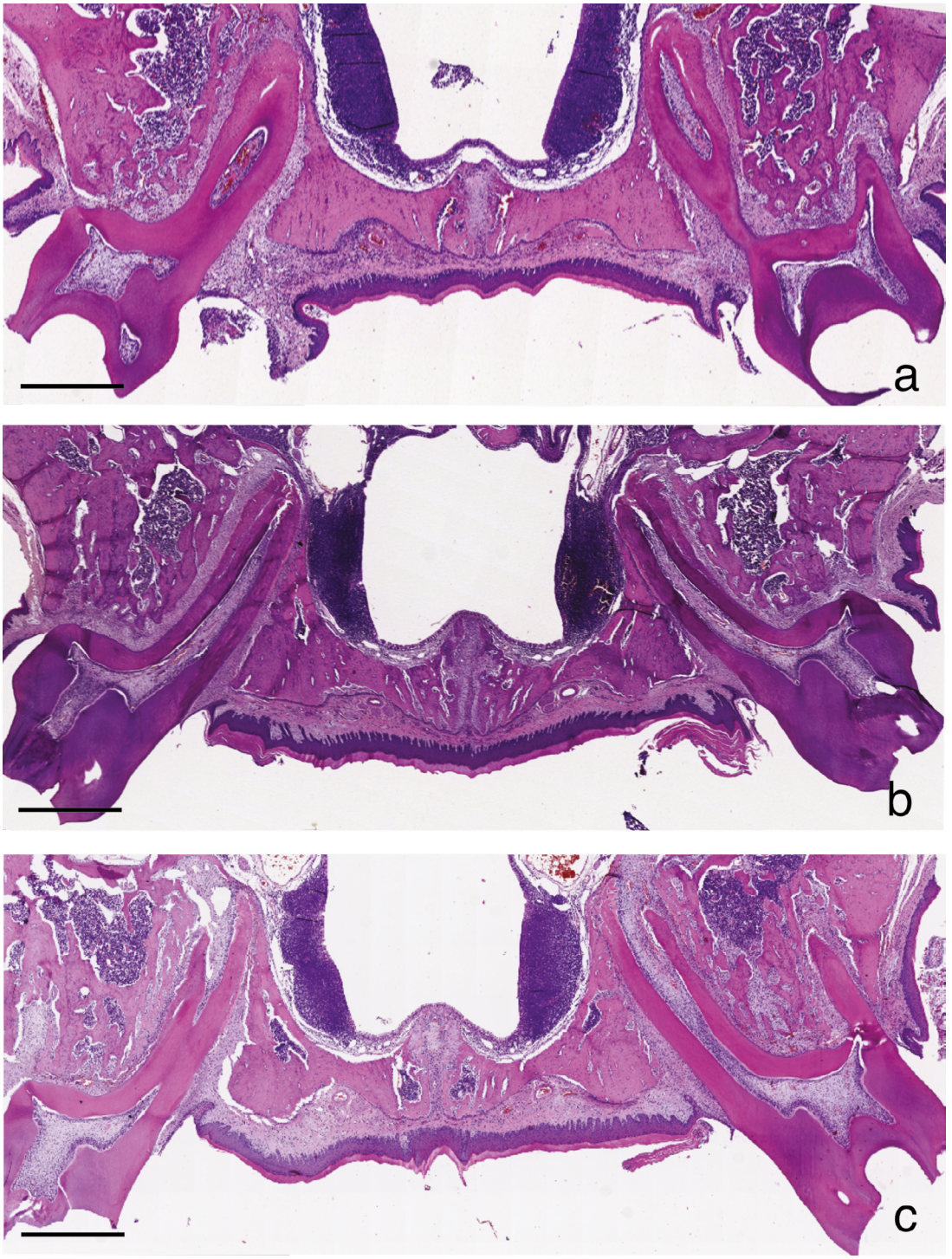

Fig. 3: Midpalatal suture frontal sections stained with H&E.
a-c) Histological changes in the cartilage of the midpalatal suture after force application. Frontal photomicrographs of rat mid-palatal suture in a control animal (a) and animals subjected to 60g (b) and 90g (c) expansive forces. In the expanded groups, greater intermolar width and a larger suture area were observed in relation to the control group. Stained with H&E, original magnification 10X. Scale 1000 µm.

area was significantly greater in group E60 than in group C, and the fibrous area associated with the suture was significantly greater in both experimental groups, E60 and E90, than in C (Fig. 5f-g).

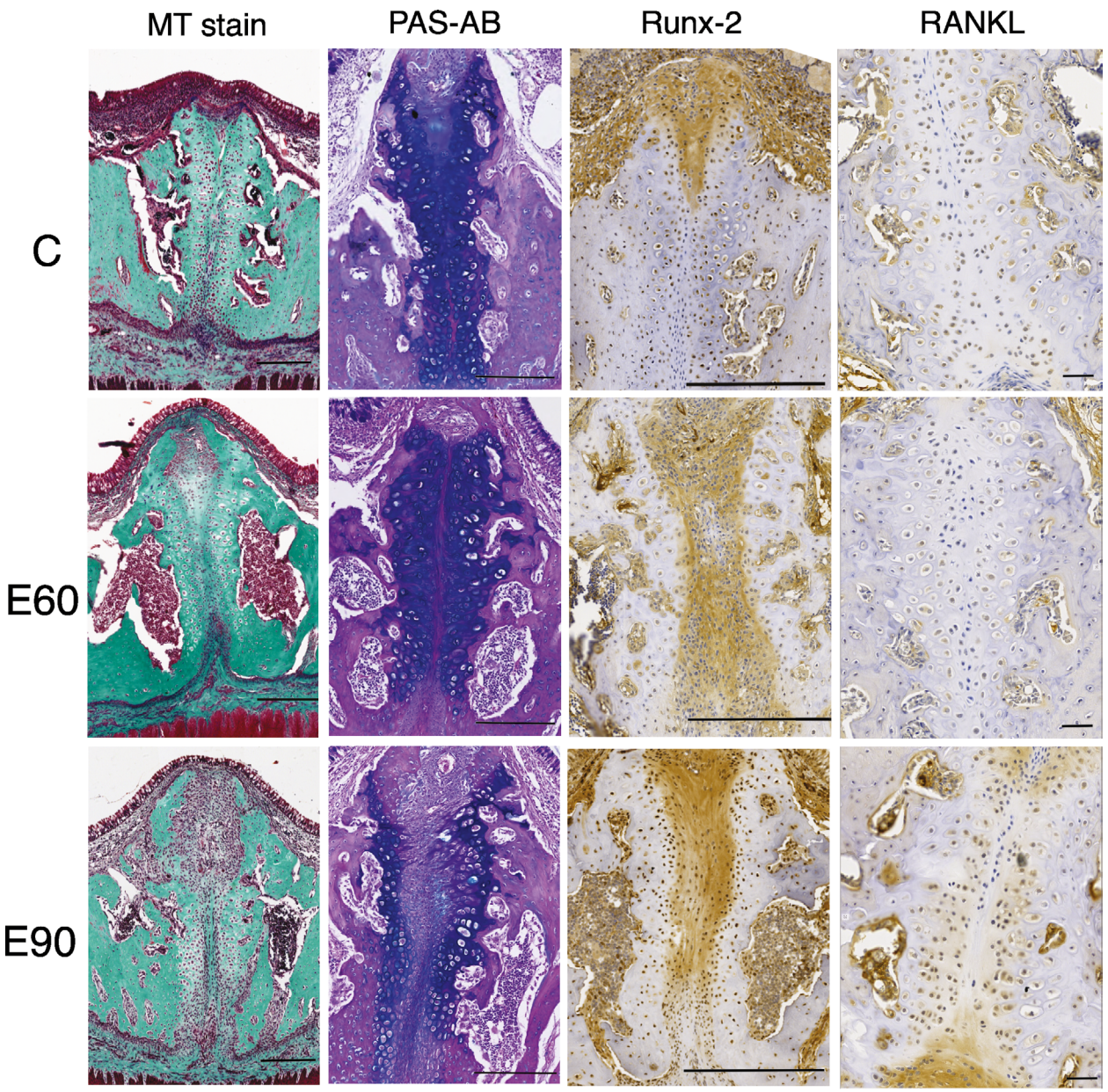

Fig. 4: Histological and immunohistochemical analysis of midpalatal sutures.
MT stain: Masson's trichrome stain. Images show palatal process bone tissue in turquoise, epithelium and connective tissue in red, and suture cartilaginous tissue in light green. Experimental animals (E60 and E90) show an increase in suture area, and greater fibrous tissue infiltrate within the suture. Original magnification 20X. Scale 200 µm.
PAS-AB: The images show bone tissue in pink, (PAS+, neutral mucins) periphery of suture cartilage in fuchsia, and (AB+, acid mucins) central portion of the cartilaginous matrix of the suture in blue. Animals subjected to expansive forces (E60 and E90) show an increase in suture area and width, with changes in the distribution of cartilaginous matrix components, displaying an increase in PAS+ matrix. Periodic Acid-Schiff-Alcian blue stain. Original magnification 20X. Scale 200 µm.
Runx-2 immunohistochemistry: Photomicrographs of midpalatal sutures of animals from groups C, E60 and E90 with Runx2 immunodetection, showing an increase in Runx2 positive mesenchymal-like cells in the experimental groups (E60 and E90) compared to the control. Original magnification 10X. Scale 300 µm.
RANKL immunohistochemistry: Photomicrographs at higher magnification of midpalatal sutures of animals in groups C, E60 and E90 with immunodetection for RANKL, clearly showing the smaller quantity of RANKL+ chondrocytes in animals from group E60 compared to animals from groups C and E90. Original magnification 40X. Scale 50 µm.

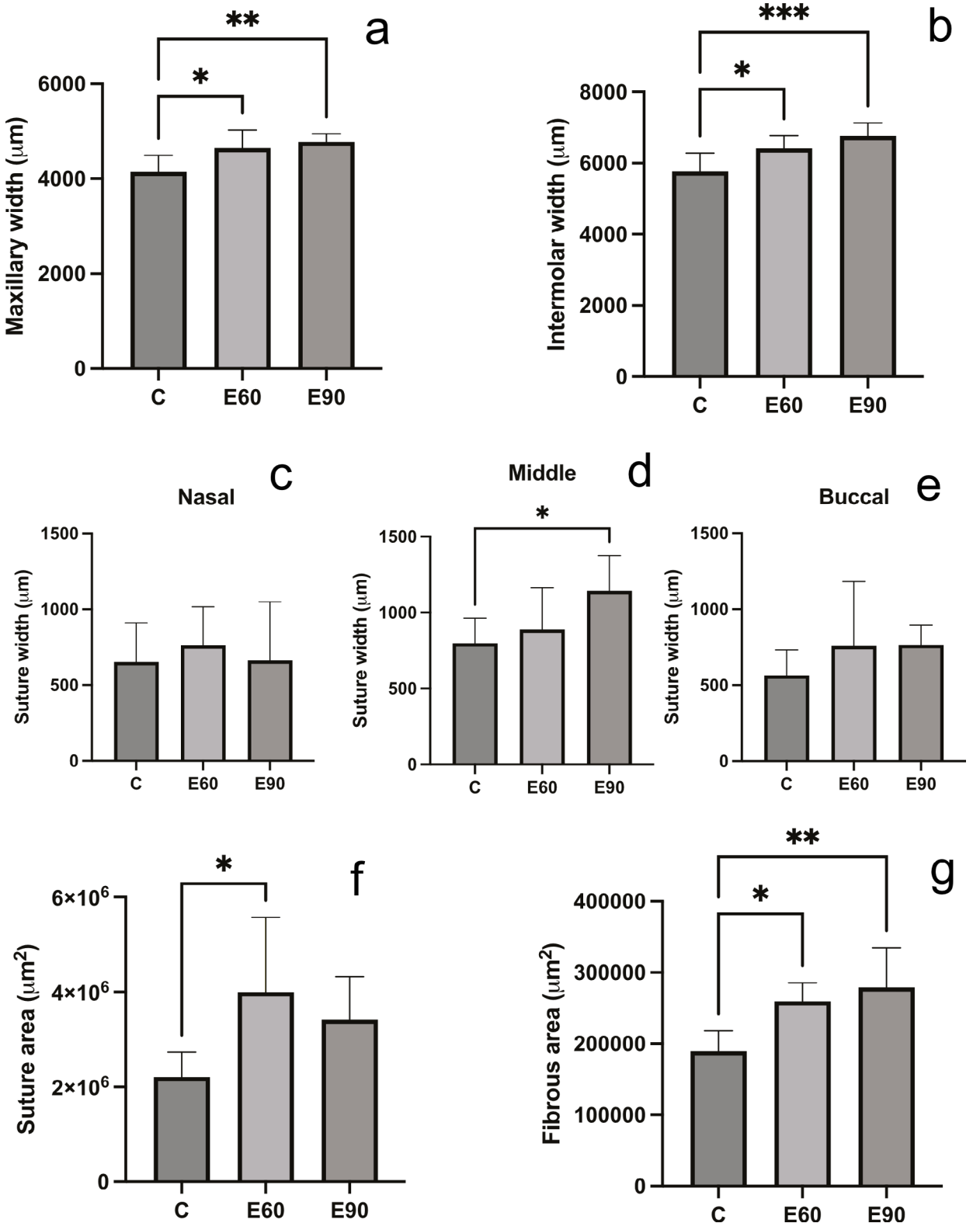

Fig. 5: Histomorphometric evaluation.
*a) Maxillary width, b) Intermolar width, c) Suture width at nasal level, d) Suture width at middle level, e) Suture width at buccal level, f) Suture area, g) Fibrous area inside the cartilage of the suture. Results are expressed as mean±SEM, n=5-7 animals per group. One-way ANOVA in conjunction with Tukey's post-test were performed, *p<0.05, **p<0.01, ***p<0.001.*

The percentage and number of Runx2+ chondrocytes/mm^2 did not differ significantly among groups (Fig. 6a-b). However, the percentage and number of Runx2+mesenchymal-like cells/mm^2 were significantly greater in the two experimental groups than in the control (Fig. 6c-d). The number of RANKL+ chondrocytes/mm^2 was significantly lower in the suture of the animals in group E60 than in groups C and E90, which did not differ significantly from each other (Fig. 6e).

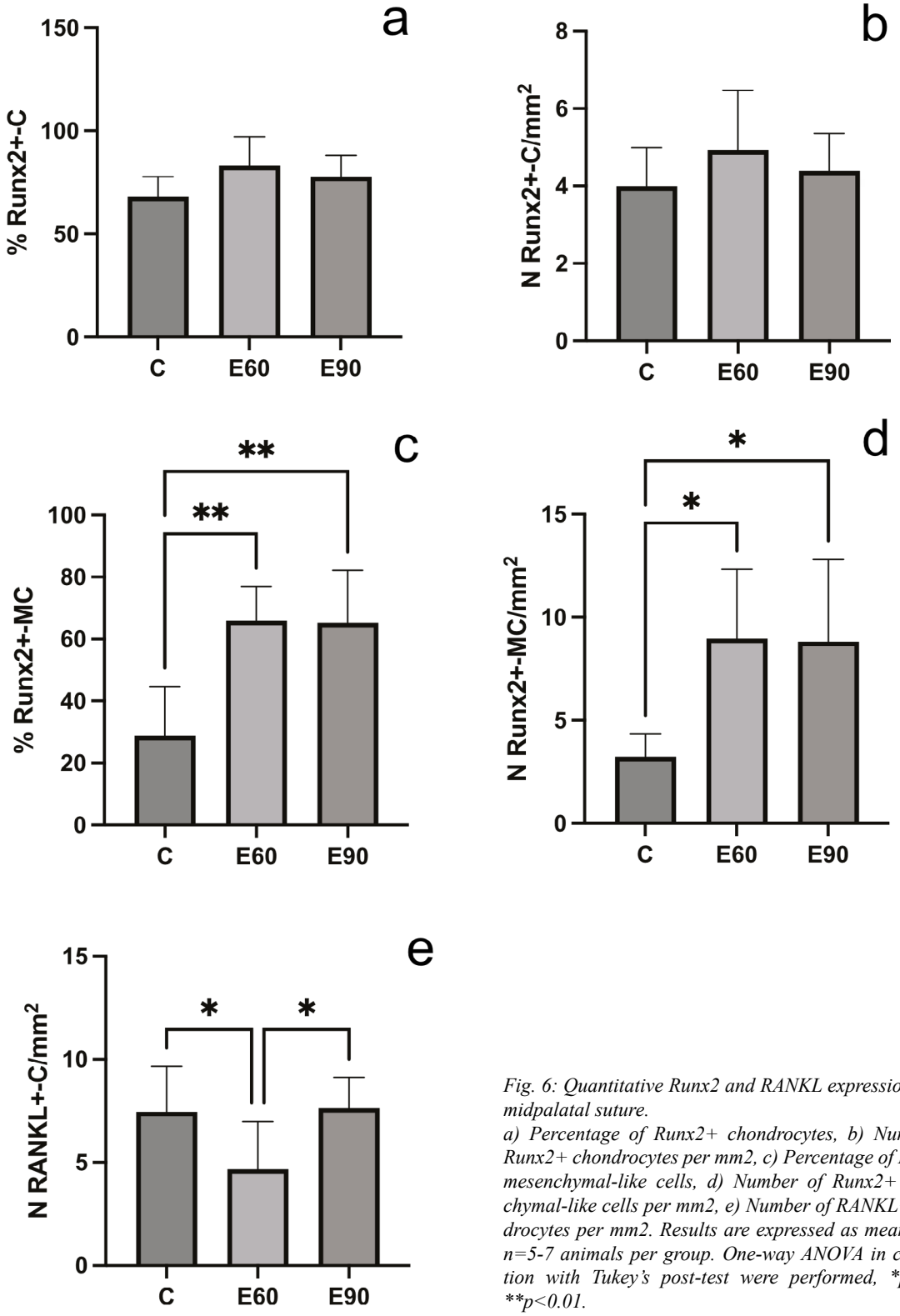

Fig. 6: Quantitative Runx2 and RANKL expression in the midpalatal suture.
*a) Percentage of Runx2+ chondrocytes, b) Number of Runx2+ chondrocytes per mm2, c) Percentage of Runx2+ mesenchymal-like cells, d) Number of Runx2+ mesenchymal-like cells per mm2, e) Number of RANKL+ chondrocytes per mm2. Results are expressed as mean±SEM, n=5-7 animals per group. One-way ANOVA in conjunction with Tukey's post-test were performed, *p<0.05, **p<0.01.*

DISCUSSION

Sutures allow stress distribution and bone remodelling during growth through sutural distraction osteogenesis. Palatal expansion through midpalatal suture distraction achieved by applying tensile forces is the most effective treatment for correcting maxillary constriction. Since most of the mechanisms involved are not yet fully understood, especially in relation to the magnitude of the force applied, it is crucial to investigate the intrinsic mechanisms that drive ossification of the suture as a function of the application of forces of different magnitudes. The present study tested expansive forces of different magnitudes, and found that when the forces were within a physiological range that did not generate inflammation or areas of hyalinosis/necrosis in the expanded zone, the degree of suture expansion was similar, with active participation of key molecules responsible for modulating the bone remodelling process. However, considering that the responses were similar, maxillary expansion with light, continuous force is the option of choice because the molars maintained healthy periodontal support as the alveolar process was expanded[19]. Other authors emphasize the use of light forces to promote chondrocyte proliferation and induce a better suture cartilage response pattern compared to stronger expansion[11].

In rat midpalatal suture, suture cartilage ossification has generally been observed as endochondral bone formation at the boundary between the maxillary bone and the cartilage[8,11,19,20]. However, regarding ossification, the results of histological and histomorphometric observations indicate the invasion and proliferation of fibrous tissue from the periosteum, with the presence of abundant mesenchymal-like cells, suggesting that intramembranous bone formation is induced at the boundary between the layers of cartilage following expansion. These observations are consistent with the descriptions in the first studies of maxillary expansion in rats[9], in which the application of tensile force to the midpalatal suture cartilage changed the phenotypic expression of osteochondroprogenitor cells in the secondary cartilage, indicating pathway differentiation. The tensile forces applied during expansion induce the formation of new bone in the mid-palatal suture, and there is suture remodelling throughout the expansion procedure, involving bone resorption, bone formation and fibre reorganization[21,22].

According to the above, the increase Runx2 expression in mesenchymal-like cells in the expanded midpalatal suture suggests that the osteochondroprogenitor cells differentiated into preosteoblasts in response to the expansive force. Runx2 is considered an essential transcription factor for osteoblast differentiation, which is determinant in early osteogenesis[16]. In the midpalatal suture samples, the expression of osteoblast markers, including Runx2, increased significantly in the expanded suture tissues as determined by qPCR at 7 and 14 days, suggesting an increase in osteoblastic differentiation and activity[14]. Yi et al.[23] reported similar results at 7 days of 50g expansive force application. The current study found increased Runx2 expression in mesenchymal-like cells, which is related to direct differentiation into osteoblasts in the initial phase of osteogenic induction. Studies by Takahashi et al.[9], published several decades ago, already suggested that the evidence of type I collagen and the decrease in cartilaginous extracellular matrix rich in type Il collagen during the early stages were caused by inhibition of the chondrogenic differentiation of progenitor cells and the differentiation into preosteoblasts in response to the expansive force in rats. In a recent study of a control suture consisting of cartilage-covered palatine connected by a thin band of fibrous tissue, it was found that after one week of expansion, there was an increase in proliferation of osteoblast-like cells, considered periosteal cells, indicating active bone formation[24]. The observations in the current study strongly suggest that rather than being endochondral, bone formation is intramembranous, occurring concomitantly with changes in the composition of the cartilaginous matrix produced by new osteoblasts derived from mesenchymal-like progenitor cells that invade the suture, arising from nearby periosteum during the earliest stages of expansion.

Receptor activator of nuclear factor-kappa B ligand (RANKL), an osteoclastogenesis regulatory molecule, plays an important role in inducing bone remodelling. Arnez et al.[13] showed that rapid maxillary expansion in rats upregulated the expression of RANKL at 3 and 7 days, and downregulated it at 10 days. Guerrero et al.[14] verified an increased expression of Rank, Rankl and Opg at suture sites under mechanical force, although they found no difference in osteoclast

numbers. Yi et al.[23] found an increase in RANKL expression immunohistochemically at 7 days with a 50g expansive force, resulting in enhanced osteoclast differentiation and activation during expansion. Similar results were reported by Chen et al.[15], where RANKL and OPG increased after rapid maxillary expansion. Hypertrophic chondrocytes are a source of the RANKL required to induce osteoclastogenesis and formation of the marrow space during endochondral ossification. Indeed, in Rankl$^{-/-}$ knockout mice, calcified cartilage resorption was prevented by reducing RANKL expression in hypertrophic cells[25]. In our study, the number of RANKL+ chondrocytes/mm^2 was significantly lower in group E60 than in groups C and E90, suggesting that lighter expansive forces would reduce osteoclast recruitment and differentiation towards the interior of the suture cartilage, inhibiting bone resorption and preventing its endochondral ossification.

Human and rat midpalatal suture differ mainly in structure and growth. While both serve as palatal growth centres, rat midpalatal suture contains secondary cartilage that will be replaced by endochondral ossification[6], in contrast to the intramembranous ossification that occurs in the humans[1]. Nevertheless, the rat model is useful and valuable for studying the maxillary expansion and bone remodelling mechanism, particularly in relation to bone formation and the analysis of osteoblast response.

CONCLUSIONS

The reduction in RANKL expression in chondrocytes and the increase in Runx2 expression in mesenchymal-like cells after expansion suggest that lighter expansive forces would stimulate osteogenesis in a direct manner not associated to the endochondral ossification of the secondary cartilage present in the suture. The results of this study contribute novel data on cell response to expansive forces of different magnitudes, enabling partial elucidation of how the processes of bone resorption and formation involved in rapid maxillary expansion are regulated, independently of the maturation of the tissue present in the suture.

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CONFLICT OF INTERESTS

All the authors declare that there is no conflict of interest regarding the publication of this manuscript.

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Assessment of compressive and flexural properties of three contemporary bulk fill resin composites

Norma Borja-Farfán, Danilo Loyola-Livias, Leslie Casas-Apayco, Monica Hermoza-Novoa

Universidad Peruana de Ciencias Aplicadas, Carrera de Odontología, Lima-Perú

ABSTRACT

Contemporary restorative materials must demonstrate how much stress they can withstand before permanently deforming under the constant forces generated during chewing. ***Aim:*** *To compare the compressive and flexural strength of three bulk fill resin composites.* ***Materials and Method:*** *This study analyzed three bulk fill resin composites: Opus™ bulk fill (OBF), Tetric® N-Ceram bulk fill (TNC), and Filtek™ bulk fill (FBF), and one conventional resin composite: Filtek™ Z350XT (FZXT) as a control group. Each composite was used to prepare 14 cylindrical specimens (n=56) for compressive strength tests, and 19 rectangular specimens (n=76) for flexural strength tests. Mechanical tests were performed using an Instron® universal testing machine - 100 kN load at a speed of 1 mm/1min.* ***Results:*** *Descriptive (Mean ± SD) and inferential statistics (Kruskal-Wallis test/ post-hoc test), p<0.05 were used. The compressive test results (Means ± SD) were: OBF (141.68MPa ± 22.20), TNC (139.03MPa ± 23.56), FBF (235.59MPa ± 26.08), and FZXT (99.28MPa ± 11.36). The flexural test results (Means ± SD) were: OBF (116.29 ± 7.20), TNCBF (109.67MPa ± 7.58), FBF (200.53MPa ± 10.32), and FZXT (90.08MPa ± 8.63). Statistically significant differences were found between the resin composites (p<0.001) in both mechanical tests.* ***Conclusion:*** *The bulk fill resin composites demonstrated higher compressive and flexural strength than the conventional resin composite. However, the use of these restorative materials may depend on specific clinical requirements and material handling preferences.*

Keywords: *compressive strength - composite resins - dental materials - flexural strength*

Evaluación de las propiedades compresivas y flexurales de tres resinas bulk-fill contemporáneas

RESUMEN

Los materiales restauradores contemporáneos deben demostrar una adecuada resistencia a la deformación frente a las fuerzas masticatorias constantes a las que se encuentran sometidos. ***Objetivo:*** *El objetivo de este estudio fue comparar la resistencia compresiva y flexural de tres resinas compuestas bulk fill— Opus™ Bulk Fill (OBF), Tetric® N-Ceram Bulk Fill (TNC) y Filtek™ Bulk Fill (FBF)—con una resina compuesta convencional, Filtek™ Z350XT (FZXT), empleada como grupo control.* ***Materiales y Método:*** *Se prepararon 56 especímenes circulares para la prueba de resistencia compresiva (n=14 por grupo) y 76 especímenes rectangulares para la prueba de resistencia flexural (n=19 por grupo). Las pruebas mecánicas se realizaron en una máquina universal de ensayos Instron® (capacidad de carga de 100 kN) a una velocidad de 1 mm/min. Se emplearon estadísticas descriptivas (media ± DE) y análisis inferenciales (prueba de Kruskal–Wallis con comparaciones post-hoc; α=0.05).* ***Resultados:*** *Los valores de resistencia compresiva (media ± DE) fueron: OBF: 141.68 ± 22.20 MPa, TNC: 139.03 ± 23.56 MPa, FBF: 235.59 ± 26.08 MPa y FZXT: 99.28 ± 11.36 MPa. Los valores de resistencia flexural (media ± DE) fueron: OBF: 116.29 ± 7.20 MPa, TNC: 109.67 ± 7.58 MPa, FBF: 200.53 ± 10.32 MPa y FZXT: 90.08 ± 8.63 MPa. Se encontraron diferencias estadísticamente significativas entre las resinas compuestas en ambas pruebas (p<0.001).* ***Conclusión:*** *Las resinas compuestas bulk fill mostraron valores significativamente mayores de resistencia compresiva y flexural en comparación con la resina compuesta convencional. Sin embargo, el uso de estos materiales restauradores puede depender de necesidades clínicas específicas y las preferencias en la manipulación del material.*

Palabras clave: *resistencia compresiva - resinas compuestas - materiales dentales - resistencia flexural.*

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Corresponding Author:
Leslie Casas-Apayco.
leslie.casas@upc.pe,
lesliecasas@yahoo.com

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INTRODUCTION

Conservative restorative procedures are recommended to replace the complete removal of all carious tissues in the management of dental caries. In some clinical cases, these procedures involve extensive restoration (direct or semi-direct). However, resin composite restorations can fail due to the reduced mechanical and physical properties of this type of cavity configuration. There is often a correlation between compressive strength and flexural strength, but not always in direct proportion. During chewing and mandibular movements, resin composite restorations are subject to vertical (compression) and tangential (bending or flexure) forces, so it is important that they have good short- and long-term clinical performance[1,2]. Bulk fill resin composites seem to satisfy this requirement.

Bulk fill resin composites were introduced in 2010 and can be placed in a single step up to 5 mm in depth, depending on the manufacturer's instructions. They are designed for use in wide and deep cavities, eliminating the need for an additional capping layer. Bulk-fill resin composites provide several advantages, including ease of handling, time efficiency during clinical procedures, reduced risk of errors in restorative applications, minimized polymerization shrinkage, and decreased cuspal deflection[2,3]. They can be applied in ≥ 4 mm increments[4,5], and contain photoinitiators such as Ivocerin and Camphorquinone, which can be activated through high-intensity light (up to 1000 nm) that enables additional light penetration to the dentin at the bottom of the cavity.

Given the increasing interest in bulk-fill resin composites, the aim of this study was to compare the compressive and flexural strength of three bulk fill resin composites and one conventional resin composite.

MATERIALS AND METHOD

This was an *in vitro* study with experimental design. It was approved by the Ethics Committee of Faculty of Health Sciences of Universidad Peruana de Ciencias Aplicadas (Number PI 121-17)

The following composites were analyzed and divided in groups according to each test: Opus™ bulk fill *(OBF)*, G2- Tetric® N-Ceram bulk fill *(TNC)*, Filtek™ bulk fill *(FBF)*, and Filtek™ Z350XT *(FZXT)* conventional resin composite as a control group. The restorative materials used in this study are described in Table 1.

Table 1. Characteristics of the materials used in the study.

Material	Shade	Country	Manufacturer	Composition	Filler amount (Wt/Vol)	Filler size (µm)	Batch Number
Opus™ Bulk Fill **(OBF)** **G1-G5**	A2	Brazil	FGM	Urethane dimethacrylate, stabilizers, camphorquinone, co-initiator, inorganic load filler of silanized silica dioxide, stabilizers and pigments.	79% (wt)	10 - 0.7 µm	030817
Tetric® N-Ceram Bulk Fill® **(TNC)** **G2-G6**	IVA	Liechtenstein	Ivoclar Vivadent	Dimethacrylates, Bis-GMA, Bis-EMA, Barium glass, Ytterbium trifluoride, prepolymer, mixed oxide, additives, stabilizers, catalysts and pigments.	53%-55% (vol)	0.04 -3 µm	W36673
Filtek™ Bulk Fill **(FBF)** **G3-G7**	A2	USA	3M ESPE	Silica, zirconia, ytterbium trifluoride, AUDMA, UDMA, AFM, 1, 12-dodecane-DMA	58.4% (vol)	0.004 -0.1 µm	N857430
Filtek™ Z350XT **(FZXT)** **G4-G8**	A2	USA	3M ESPE	Silica, zirconia, bis-GMA, UDMA, TEGDMA, and bis-EMA.	55.6% (vol)	0.6 - 20 µm	N741410

Compressive and flexural tests

To test compressive strength, 14 cylindrical specimens (3 mm x 5 mm) were prepared from each composite (n=56) (G1 to G4). To test flexural strength, 19 rectangular specimens (2 mm x 2 mm x 25 mm, ISO 4049-2000) were prepared from each composite (n=76) (G5 to G8).

The bulk fill resin composites were placed in molds using the single increment technique (5 mm), and the conventional resin composite was placed using the oblique incremental technique (2 increments/2 mm). The curing time was selected according to manufacturer's indications, and performed using a LEDdition® (Ivoclar Vivadent, Liechtenstein, 850mW/cm^2- OBF: 40 sec/5mm, TNC: 20 s /5 mm, FBF: 40 s /5 mm and FZXT: 20 s/2 mm). Compressive test specimens were cured up to 1 cm below the outer surface layer. Flexural test specimens, due to their size, were light-cured at three consecutive points (right side, middle area, and left side). Subsequently, all specimens were stored at 37 +/- 5 °C in an oven (Hotpack® 355381 - USA) for 24 hours.

The compressive and flexural strength of the specimens were tested using an Instron® universal testing machine (Model 3382 -USA). For the compressive test, the specimen was placed vertically between two compression plates, which were connected to the load measuring cell with a constant 100 kN load applied at a crosshead speed of 1 mm/1 min until the specimen fractured. For the flexural strength test, the specimen was placed horizontally on the three-point bending device (Code OA41, ODEME, Brazil) at a crosshead speed of 1 mm/1 min until the specimen fractured. The data analysis was performed using descriptive (Mean ± SD) and inferential statistics (Kruskal Wallis test / post hoc test; $p<0.05$) for group comparisons.

RESULTS

Table 2 provides the mean values (MPa) of the compressive and flexural strengths of the experimental groups, and the comparisons using Kruskal Wallis tests (*post hoc* test). Statistically significant differences were found among the bulk resin composites ($p<0.01$) and compared to the conventional resin composite. Among the bulk fill resin composites, Filtek™ Bulk Fill showed the highest mean values of compression and flexural strength, followed by Opus™ Bulk Fill and finally Tetric® N-Ceram Bulk Fill® ($p<0.05$; Table 2).

DISCUSSION

Compressive and flexural strength are key properties for dental composites, reflecting their ability to withstand chewing forces without permanent deformation. In this study, all three bulk-fill resins – Opus™ Bulk Fill (OBF), Filtek™ Bulk Fill (FBF), and Tetric® N-Ceram Bulk Fill (TNC) – performed better than the conventional resin composite. Filtek™ Bulk Fill consistently demonstrated the highest values of compressive and flexural strength. These results align with previous studies and highlight the advantages of bulk-fill materials for posterior restorations[1,3,4,6-8].

The strong mechanical behavior of these materials is largely provided by their composition. Higher filler content and smaller particle sizes increase stiffness and strength, while high-molecular-weight monomers such as UDMA, AUDMA, and bis-EMA help reduce polymerization shrinkage and stress, creating a more stable, cross-linked polymer network[1,4,6,8]. In addition, AFM (Addition–Fragmentation Monomer) acts as a stress-relieving agent by allowing reversible bond cleavage during polymerization, particularly in deeper layers, which helps maintain both compressive and flexural

Table 2. Comparison of compressive and flexural strength of bulk fill resin composites

Resin composites	Compressive Strength			Flexural strength		
	Mean (MPa)	SD	p*	Mean (MPa)	SD	p*
Opus™ Bulk Fill **(OBF)**	141.68 b	22.20	<0.001	116.29 b	7.20	<0.001
Tetric® N-Ceram Bulk Fill® **(TNC)**	139.03 b	23.56		109.67 b	7.58	
Filtek™ Bulk Fill **(FBF)**	235.59 a	26.08		200.53 a	10.32	
Filtek™ Z350XT **(FZXT)**	99.28 c	11.36		90.08 c	8.63	

* Kruskal Wallis test (post hoc) among all groups. Significance level ($p<0.05$).
The same letters indicate no significant statistical difference.

integrity[1,6]. Specifically, Opus™ Bulk Fill combines ~79% filler by mass with a UDMA-based matrix to dissipate stress effectively; Tetric® N-Ceram Bulk Fill uses high-molecular-weight monomers such as dimethacrylates, Bis-GMA, Bis-EMA with filler-refractive index, matching to optimize light penetration; and Filtek™ Bulk Fill further combines UDMA, bis-EMA, AFM, and AUDMA stress-relieving monomers, with an optimized zirconia/silica filler system, enhancing its compressive and flexural properties[3,7,8].

Polymerization efficiency and depth of cure are equally important. Proper light penetration and a high degree of conversion produce a stable, cross-linked network that minimizes residual monomers and stress concentration, directly influencing mechanical strength[8-10]. Bulk-fill composites are designed for increments of 4–5 mm, and their resin-filler systems, combined with refractive index matching and internal light reflection, ensure uniform curing even in deeper regions of the restoration[3-5]. Inadequate polymerization at greater depths, however, can reduce cross-linking, lowering stiffness and compressive and flexural resistance, which underscores the need for proper curing strategies, including correct light intensity, exposure time, and additional techniques for proximal and deep areas[3,6,7,10,11]. In Opus™ Bulk Fill, the urethane dimethacrylate matrix and high filler content facilitate stress dissipation, improving compressive and flexural resistance[1,7]. Filtek™ Bulk Fill benefits from high-molecular-weight monomers (UDMA, bis-EMA, AUDMA) and stress-relieving AFM, which stabilize the polymer network and allow superior depth of cure, particularly in deeper increments[1,6,8]. Tetric® N-Ceram Bulk Fill achieves adequate polymerization through high-molecular-weight monomers and optimized filler-refractive index matching, ensuring a sufficient degree of conversion in deeper regions and supporting mechanical strength[3-5]. Efficient polymerization throughout the entire increment depth is essential for maximizing compressive and flexural performance, especially in deep or extensive cavities [3-5,9,11]. Clinicians should consider complementary strategies to achieve adequate polymerization at high-increment depths in deep cavities[3,6,7,11], both on occlusal surfaces and in proximal regions, after removal of the sectional or other matrix systems.

High-intensity curing lights (>1100 mW/cm²) are optimal for bulk-fill composites, though sufficient polymerization can also be achieved with lower-intensity lamps (~850 mW/cm²) if exposure time is properly adjusted[10,11]. In essence, the combination of optimized monomer systems, stress-relieving agents, high filler loading, and effective polymerization explains the excellent mechanical performance observed in all three bulk-fill resins. Whilst Filtek™ Bulk Fill showed stronger mechanical properties than the others, Opus™ Bulk Fill and Tetric® N-Ceram Bulk Fill also demonstrated good mechanical properties, supporting them as reliable dental materials for extensive posterior restorations[1,3,6-8].

New restorative dental materials are continuously being developed, so it is of the utmost importance to constantly evaluate the mechanical properties that improve the clinical and functional behavior of extensive restorations.

CONCLUSION

The bulk fill technique can be considered an effective alternative to the incremental fill technique for restoring extensive occlusal and proximal cavities. The bulk fill resin composites tested demonstrated superior compressive and flexural strength to the conventional resin composite. Use of these restorative materials may depend on specific clinical requirements and material handling preferences.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Fluoride content in children's dentifrices marketed in Argentina

Ana Sorazabal[1], Lidia AR Valadas[1], Angela B Argentieri[1], Pablo Salgado[1], Thereza C Farias Botelho Dantas[2,3], Celiane M Carneiro Tapety[4], Aldo Squassi[1]

1. Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Odontología Preventiva y Comunitaria, Buenos Aires, Argentina.
2. Faculdade Paulo Picanço, Curso de Odontologia, Fortaleza, Brasil.
3. Centro Universitário Christus, Curso de Odontologia, Fortaleza, Brasil.
4. Universidade Federal do Ceará, Faculdade de Farmácia, Odontologia e Enfermagem, Fortaleza, Brasil.

ABSTRACT

Fluoridated dentifrices are the most widely used formulations for caries prevention and control. However, there is great concern regarding legislation on the use of fluoride formulations in early childhood from the standpoint of avoiding increased risk of fluorosis while at the same time achieving a concentration that ensures anticaries activity. ***AIM:*** *The aim of this study was to evaluate the stability of fluoride concentration in the main children's toothpaste brands marketed in Argentina.* ***Materials and Method:*** *Twenty different brands of children's toothpaste were evaluated by analyzing the concentration of total fluoride (TF), total soluble fluoride (TSF) and ionic fluoride (IF) in two or three samples of each brand. Each sample was analyzed in duplicate using an ion-specific electrode calibrated with fluoride standards, and the results were expressed in ppm (µg F/g).* ***Results:*** *TF was found to be lower than stated by the manufacturer in 85% of the brands analyzed. TF concentration found in fluoride toothpastes ranged from 651.5 to 1090.4 µg/ml F-, while TSF ranged from 504.2 to 915.5 µg/ml F, and IF from 298.6 to 838.2 µg/ml F-.* ***Conclusions:*** *The formulation and fluoride content in children's toothpastes marketed in Argentina vary greatly according to brand. In all the samples, TF concentration was lower than 1500 ppm F-, and TSF concentration was lower than 1000 ppm F-, the minimum amount necessary to ensure anticaries effect. This indicates the need for improved formulations and regulations.*

Keywords: *fluoride - toothpaste - dental caries - dental gel*

Concentración de fluoruros en pastas dentales infantiles comercializadas en Argentina

RESUMEN

Los dentífricos fluorurados son las formulaciones más utilizadas para la prevención y el control de las caries. Sin embargo, existe una gran preocupación de las legislaciones sobre el uso de formulaciones fluoradas en la primera infancia y el mayor riesgo de fluorosis, en comparación con la preocupación de una concentración que asegura una actividad anticáries. ***Objetivo:*** *El objetivo de este estudio fue evaluar la estabilidad de la concentración de fluoruro, en los principales dentífricos infantiles comercializados en Argentina.* ***Materiales y Método:*** *Se evaluaron 20 marcas diferentes de dentífricos infantiles, con análisis de la concentración de fluoruro total (TF), fluoruro total soluble (FST) y fluoruro iónico (IF) de dos o tres muestras de cada marca (µg/g/F). Cada muestra se analizó por duplicado utilizando un electrodo específico de iones calibrado con estándares de fluoruro y los resultados se expresaron en ppm (µg F/g).* ***Resultados:*** *El TF encontrado fue inferior al declarado por el fabricante en el 85% de las marcas analizadas. La concentración de TF encontrada en los dentífricos fluorados varió de 651,5 a 1090,4 µg/ml F-, mientras que el TSF varió de 504,2 a 915,5 µg/ml F y el IF de 298,6 a 838,2.* ***Conclusiones:*** *Las pastas dentales comercializadas para niños en Argentina presentan una gran variabilidad en cuanto a marca, formulación y contenido de fluoruro. La concentración de TF en las muestras fue inferior a 1500 ppm de F^- y todas presentaron concentraciones de TSF inferiores a 1000 ppm de F^-, la cantidad mínima necesaria para tener efecto anti-caries. Esto indica la necesidad de mejorar las formulaciones y las regulaciones.*

Palabras clave: *fluoruro - pasta dental - caries dental - gel dental*

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Corresponding Author:
Ana Sorazabal
ana.sorazabal@odontologia.uba.ar

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INTRODUCTION

Dental caries is a dynamic, biofilm-mediated disease caused by the production of acids by cariogenic microorganisms. Its development is modulated by dietary habits and influenced by a combination of biological, behavioral and social factors[1,2], including the anatomical characteristics of the tooth surface, salivary flow and composition, oral microbiome composition, and exposure to fluoride. Fluoride promotes remineralization and inhibits demineralization[3].

Although dental caries can affect all age groups, its consequences are particularly significant in children, in whom the disease is associated with pain, school absenteeism, emotional distress, and diminished quality of life[1]. There has been a great effort to control dental caries in recent decades, with an essential role being played by dentifrice formulations. However, other non-bacterial pathologies such as enamel defects, which are also studied in the area of cariology, require attention too[4].

Among preventive measures, fluoridated formulations – particularly toothpastes – have proven highly effective in reducing the incidence of caries. As products intended for daily oral hygiene, they deliver fluoride consistently to the oral environment, thereby supporting remineralization and inhibiting caries progression[3,5-8].

Concerns have been raised regarding the use of fluoridated toothpastes during early childhood, primarily due to the potential risk of dental fluorosis[8,9]. However, fluorosis results from chronic excessive fluoride ingestion during enamel formation, regardless of the fluoride source, and not from appropriate topical use[8,10]. Although caries and dental fluorosis both require attention, it is important to highlight that the critical period for fluorosis development is limited to the first 6–8 years of life, whereas caries risk persists throughout the individual's lifetime, beginning with the eruption of the first tooth. Caries leave consequences and increase healthcare costs[11-12].

Fluoride toothpaste should be associated with tooth brushing as from the eruption of the first tooth. Considering both caries prevention and the risk of fluorosis, toothpastes with 1,000 to 1,500 ppm of total fluoride are recommended for children, with at least 1,000 ppm in soluble form[6,13-15]. However, many toothpastes intended for children contain low fluoride concentration and therefore lack anti-cavity efficacy[7]. These may only be recommended for children under 3 years of age with a low risk of cavities and a high risk of developing fluorosis in the permanent upper central incisors due to regular exposure to high fluoride intake from sources other than fluoridated toothpaste, such as food, especially if they live in an area with fluoridated water[5].

Due to concerns about the risk of fluorosis, Mercosur legislation only prioritizes the safety of fluoridated toothpaste to the detriment of its anti-caries potential, as it only establishes that fluoride (F) concentration in the toothpaste shall not exceed 1,500 ppm, without establishing the soluble fluoride concentration[14]. Therefore, some toothpastes on the market contain more than 1,000 ppm (F), but with a high concentration of insoluble salts, and several others are formulated with less than 1,000 ppm F because it is allowed by legislation, which is not in accordance with the best current scientific evidence[6,9,14-16] .

The aim of this study was to determine the fluoride content in toothpaste brands recommended for children in Argentina.

MATERIALS AND METHOD

Sampling

A survey was conducted through websites, supermarkets and pharmacies to identify all commercially available toothpaste brands intended for children in Buenos Aires, Argentina. A total of 13 brands and 20 different formulations were identified and acquired from various retail sources. For each brand, three distinct lots within the expiration date were purchased. All products were included in the analysis. Each sample was encoded, and the analysis sequence was randomized using Microsoft Excel to ensure blind evaluation (Table 1).

Determination of fluoride concentration

Fluoride concentrations were determined following the protocol described by Cury et al.[17]. Each sample was analyzed in duplicate, where 90 to 110 mg (± 0.01 mg) of each sample were weighed and homogenized under vigorous stirring with 10 mL of distilled water until a suspension was obtained. Two 0.25 mL aliquots of each suspension were transferred to test tubes to determine the concentration of total fluoride (TF) and soluble fluoride. The remaining suspension was centrifuged at 3,000 g for 10 minutes at room

Table 1. Dentifrices for children marketed in Argentina and analyzed in this study

Code	Brand	Country	Lot1 Lot2 Lot3	Abrasive stated	Source of (F)	Total Fluoride stated (ppm or %)	Other information stated
A	**BUCALTAC**	Argentina	06/24 07/23 07/25	$CaCO_3$/ Hydrated Silica	MFP	700	
B	**CHICCO Niños 6-24 meses**	Italy	0POL5053	Hydrated Silica	NaF	1000	Xilitol
C	**CREST Kids**	Greece USA	0434GR 31984354P1	Hydrated Silica	NaF	0,243%	
D	**COLGATE Kids**	Brazil	2277BR122K 2195BR121C 2185BR122C	Hydrated Silica	NaF	1100	
E	**COLGATE Kids Zero**	Mexico	1277MX111H 2039MX112H 1242MX111H	Hydrated Silica	NaF	1100	
F	**COLGATE Smiles**	Mexico	2206MX1116 2132MX1126 2036MX1116	Hydrated Silica	NaF	1100	
G	**COLGATE Teens**	Brazil	153BR122G 2034BR122K 2230BR121G	Hydrated Silica	NaF	1100	
H	**ELGYDIUM Niños 2/6 años**	France	G00015 G00005 G00008	Hydrated Silica	Amine fluoride	500	Silyglicol
I	**ELGYDIUM niños 3/6 años**	France	G00043 G00039	Hydrated Silica	Amine fluoride	1000	Silyglicol
J	**ELGYDIUM Junior 7/12 años**	France	G00036 G00033 G00011	Hydrated Silica	Amine fluoride	1400	Silyglicol
K	**FARMACITY**	Argentina	11011 30711 05521	$CaCO_3$/ Silica	MFP/ NaF	700	
L	**FLUOROGEL Chiquitos**	Argentina	14585 14577 14589	Hydrated Silica	NaF	543	Xilitol 10%
M	**FLUOROGEL Junior**	Argentina	02604 02605 02602	Hydrated Silica	NaF	1086	Xilitol 10%
N	**FRESH DENT Kids**	unknown	220405 220102 220407	$CaCO_3$/ Hydrated silica	MFP/ NaF	1000	
O	**GUM**	Argentina	220921 220307 220516	Hydrated Silica	NaF	1100	
P	**JUNIOR SMILE (Dia)**	Argentina	09521 11211 24321	$CaCO_3$/ Hydrated silica	MFP NaF	700	
Q	**ODOLITO**	Brazil	2221BR122C 2095BR122C 2221BR121C	Hydrated silica	NaF	1100	
R	**ORAL B Kids**	Mexico	21434354P0	Hydrated silica	NaF	1100	

(continue on next page)

Table 1. Dentifrices for children marketed in Argentina and analyzed in this study (cont.)

S	**ORAL B Stages**	Mexico	2146435402 2192435401 2172435401	Hydrated silica	NaF	1100	
T	**SENSODYNE PRO-ESMALTE Niños**	Slovaquia	20502KWC 13092KWA 11032KWC	Hydrated silica	NaF	1100	

Sample information: code, brand, country of origin, lot numbers, abrasive substance stated, source of (F), total fluoride stated (ppm or %) by manufacturers and other information stated.

temperature to separate the insoluble fluoride bound to abrasives.

For each lot, two 0.25 mL aliquots of the supernatant were transferred to test tubes to determine the concentration of total soluble fluoride (TSF) and to other tubes to determine the ionic fluoride (IF) concentration. All test tubes received 0.25 mL of 2.0 M HCl. TF and TSF tubes were incubated at 45 ºC for 1h. All samples were neutralized by adding 0.5 mL of 1.0 M NaOH and buffered with 1 mL of TISAB II (acetate buffer, pH 5.0, containing 1.0 M NaCl and 0.4% CDTA), and analyses were performed to determine the fluoride content.

Specimens were analyzed using the potentiometry method with an ion-specific electrode (Orion 96-09) coupled to an ion analyzer (Orion EA-740; Orion Research). Prior to analysis, the electrode was calibrated using standard fluoride solutions with final concentrations of 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 µg/mL (ppm F).

Data analysis

A linear regression equation correlating the logarithm of fluoride concentration in the standards with the measured electrode potential (mV) was calculated for each calibration ($r^2 > 0.999$) using GraphPad Prism software. This equation was applied to determine the fluoride concentration in each sample, expressed in parts per million (ppm or µg/g F).

RESULTS

This study analyzed 720 aliquots corresponding to 56 samples (1-3 lots per brand) of 20 brands of dentifrices for children (Table 1). Sixty-five percent were formulated with sodium fluoride (NaF), 5% were formulated with sodium monofluorophosphate (MFP) alone, 15% combined NaF with MFP, and 15% used amine fluoride as the active agent. Regarding abrasive components, 80% contained hydrated silica, and the other 20% had a mixture of $CaCO_3$ and hydrated silica.

In 85% of the evaluated brands, the measured total fluoride (TF) was significantly lower than the amount stated by the manufacturer. Only three brands – H, I and K – had TF concentrations exceeding their labeled values.

The concentration of TF found in the fluoride dentifrices ranged from 651.5 to 1090.4 µg/ml F^-, TSF ranged from 504.2 to 915.5 µg/ml F, and IF ranged from 298.6 to 838.2 (Table 2).

All evaluated samples exhibited total soluble fluoride (TSF) levels below 1000 ppm, the minimum threshold generally accepted for effective anti-caries action.

There were statistically significant differences between the TF stated on the label and the TF found in our study in all samples except samples B and I. Interestingly, in sample R, TF and TSF did not differ significantly (Table 2).

DISCUSSION

This study aimed to evaluate the fluoride content in children's toothpastes marketed in Argentina by assessing total fluoride (TF), total soluble fluoride (TSF) and ionic fluoride (IF), which are crucial for understanding the potential anti-caries effectiveness of these formulations.

Regarding geographic origin, 40% of the products were manufactured in Argentina, followed by Mexico (20%), France (15%), Slovakia (5%), Greece (5%) and Italy (5%). These origins may involve differences in regulatory frameworks, manufacturing standards, or formulation practices that could influence fluoride content and availability. Previous studies on fluoride concentrations in dentifrices have primarily focused on products intended for adult use. In Argentina, for example, available data from Cury et al.[18] and Valadas et al.[16] have assessed adult formulations.

Table 2. Concentration (µg/ml F-) of total fluoride stated and TF, TSF and IF found in the analysis of dentifrices sold in Argentina

Dentifrice label information (µg/ml F)	Fluoride (µg/ml F)	Mean	CI 95% (LL-UL)	*p-value Student one-sample test*
A (700)	**TF***	651.5	627.1 - 675.9	<0.001
	TSF	566.1	511.8 - 620.3	
	IF	471.9	409.7 - 533.9	
	TF-TSF*	85.5	23.2 - 147.7	0.012
B (1000)	**TF**	961.3	771.4 - 1151.1	0.662
	TSF	711.7	626.6 - 796.6	
	IF	556.8	464.7 - 648.8	
	TF-TSF*	249.6	122.1 - 377.1	0.001
C (1500)	**TF***	772.3	731.6 - 812.8	<0.005
	TSF	504.2	498.7 - 509.6	
	IF	466.6	456.5 - 476.5	
	TF-TSF*	268.1	225.6 - 310.6	0.001
D (1100)	**TF***	989.7	823.5 - 1155.9	<0.005
	TSF	772.4	739.1 - 805.7	
	IF	709.8	694.4 - 725.1	
	TF-TSF*	217.3	71.9 - 362.7	0.007
E (1100)	**TF***	891.2	845.6 - 936.9	<0.001
	TSF	713.7	677.4 - 749.9	
	IF	447.1	353.6 - 540.5	
	TF-TSF*	177.5	117.5-237.6	0.001
F (1100)	**TF***	880.9	819.3 - 942.4	<0.001
	TSF	508.6	474.7 - 542.5	
	IF	298.6	187.7 - 409.4	
	TF-TSF*	372.3	335.3 - 409.2	<0.001
G (1100)	**TF***	954.2	916.4 - 991.9	<0.001
	TSF	760.5	711.7 - 809.3	
	IF	680.2	614.9 - 745.3	
	TF-TSF*	193.6	144.2 - 243.1	<0.001
H (500)	**TF***	871.2	803.4 - 938.8	<0.001
	TSF	653.7	553.4 - 753.9	
	IF	496.0	478.1 - 513.8	
	TF-TSF*	217.4	116.4 - 381.5	<0.001
I (1000)	**TF**	1058.6	987.5 - 1129.7	>0.05
	TSF	764.7	711.6 - 817.6	
	IF	597.9	506.9 - 688.8	
	TF-TSF*	293.9	271.1 - 316.7	<0.001
J (1400)	**TF***	1090.4	1054.5 - 1126.2	<0.001
	TSF	868.8	801.2 - 936.3	
	IF	758.4	680.1 - 836.8	
	TF-TSF*	221.6	133.6 - 309.6	<0.001

(continue on next page)

Table 2. Concentration (µg/ml F-) of total fluoride stated and TF, TSF and IF found in the analysis of dentifrices sold in Argentina (cont.)

K (700)	**TF***	849.0	804.6 - 893.4	<0.001
	TSF	770.0	702.8 - 837.1	
	IF	472.4	280 - 664.8	
	TF-TSF*	79.0	35.8 - 122.1	0.002
L (543)	**TF***	977.0	952.1 - 1001.9	<0.001
	TSF	775.2	756.3 - 794.1	
	IF	368.4	230.6 - 506.1	
	TF-TSF	201.8	161.9 - 241.8	
M (1086)	**TF***	855.6	831.6 - 879.5	<0.001
	TSF	829.1	800.1 - 858.1	
	IF	767.1	752.7 - 781.4	
	TF-TSF*	26.4	4.8 - 48.2	0.021
N (1000)	**TF***	837.1	809.2 - 864.9	<0.001
	TSF	796.8	780.3 - 813.2	
	IF	760.6	735.9 - 785.2	
	TF-TSF*	40.3	8.7 - 71.8	0.017
O (1100)	**TF***	975.1	951.9 - 998.3	<0.001
	TSF	910.7	896.7 - 924.7	
	IF	833.2	784.7 - 881.7	
	TF-TSF*	64.4	32.2 - 96.6	0.001
P (900)	**TF***	890.4	846.5 - 934.3	<0.001
	TSF	843.6	818.3 - 868.8	
	IF	815.8	779.3 - 852.2	
	TF-TSF*	46.8	2.4 - 91.3	0.04
Q (1100)	**TF***	899.7	861.6 - 937.7	<0.001
	TSF	813.5	802.1 - 824.9	
	IF	786.4	726.8 - 845.9	
	TF-TSF*	86.2	43.9 - 128.5	0.001
R (1100)	**TF***	911.0	896.3 - 925.5	<0.001
	TSF	915.5	902.1 - 928.8	
	IF	838.2	823.9 - 852.3	
	TF-TSF	-4.5	-24.9 - 15.8	0.635
S (1100)	**TF***	892.9	853.1 - 932.8	<0.001
	TSF	853.8	832.7 - 874.7	
	IF	824.6	811.2 - 837.9	
	TF-TSF*	39.1	6.4 - 71.9	0.023
T (1100)	**TF***	968.5	935.9 - 1001.1	<0.001
	TSF	896.3	846.2 - 946.4	
	IF	784.7	762.3 - 807.1	
	TF-TSF*	72.2	28.6 - 115.7	0.004

Abbreviations: IF, ionic fluoride; TF, total fluoride; TSF, total soluble fluoride.
*Statistical significative differences (p <0.05)

It is important to note that children's dentifrices contain flavoring agents to make them more appealing by improving taste and scent. These components can also provide breath-freshening benefits by masking odors and creating cooling or warming sensations. However, rather than merely encouraging regular use, pleasant flavors in children's toothpaste may increase the risk of accidental ingestion[19,20]. This is of particular concern because during the enamel development phase, children younger than 8 years old are at risk of developing dental fluorosis, a condition that causes enamel discoloration and, in severe cases, affects enamel structure[21,22].

Therefore, current guidelines recommend that adults and caregivers supervise children's toothbrushing using fluoridated toothpastes with standard concentrations (1,000–1,500 ppm F), while ensuring that only age-appropriate amounts are applied[12].

The above highlights the importance of knowing the amount of fluoride content in each toothpaste, especially in those marketed for children. In its 2022 consensus statement, the International Association of Paediatric Dentistry (IAPD)[23] recommended brushing children's teeth twice daily with fluoridated toothpaste containing 1,000–1,500 ppm of fluoride. The guideline emphasizes adjusting the amount of toothpaste to the child's age ("smear" for children under 3 years; "pea-sized" for ages 3–6), rather than reducing fluoride concentration. Even with the recommendation to reduce the quantity and not the concentration of toothpastes, 25% of toothpastes in Argentina contain less than 1,000 ppm. There is evidence suggesting that using low-fluoride toothpastes in children increases the risk of caries in primary teeth[13].

Anticaries activity is related to the available soluble fluoride concentration, which must be at least 1000 ppm. While laws in USA, Canada, Australia and Chile require dentifrice labels to specify the concentration of soluble fluoride, Argentina follows Mercosur legislation[14], which only establishes that the maximum concentration of TF in a toothpaste should be 1500 ppm, whether for adults or children. The same is true in Brazil and Mexico[6].

In the current study, in 100% of the samples, the soluble fluoride concentration was lower than the 1000 ppm suggested by scientific evidence. The total fluoride (TF) levels in 85% of the analyzed brands were lower than those stated by the manufacturer, except for Dentifrices *Elgydium niños 2/6 años,* *Elgydium niños 3/6 años* and *Farmacity*, which contained higher levels than stated. However, even when the concentrations were higher than stated, they were still well below the 1,500ppm limit established by Mercosur legislation.

Several of the evaluated brands have also been assessed in previous studies conducted in Latin America. For example, the values we found for brands E and F were much lower than those reported by Chavez et al.[15] in Peru, and Leite Filho et al.[6] in Brazil and Mexico. The concentration we found in brand F was also lower than those reported by Loureiro et al.[24] in Uruguay and Valadas et al.[9] in Brazil. Brands R, S, D and G in our study presented slightly lower fluoride values than those reported by Valadas et al.[9], and brands D, G and H contained lower concentrations than those reported by Leite Filho et al.[6] in Brazil. Brands C, D, R and S in our study contained lower concentrations than samples from Mexico reported by Leite Filho et al.[6]. On the other hand, brand O was found to have levels similar to those reported by Valadas et al.[9].

The findings suggest that most children's dentifrices marketed in Argentina do not meet the stated fluoride content, potentially reducing their effectiveness in preventing dental caries. The presence of lower soluble fluoride levels highlights the need for regulatory revisions to ensure compliance with scientific recommendations and maximize anti-caries benefits.

This study did not include an analysis of the chemical interaction between fluoride and the abrasive used in each toothpaste, which can affect fluoride solubility and bioavailability. Future research should explore how specific abrasive components influence fluoride stability over time, particularly after product exposure to air or during repeated use, to better simulate real-world conditions. Additionally, long-term stability studies could help determine whether the soluble fluoride content declines over the shelf life of these products.

CONCLUSIONS

Children's toothpastes marketed in Argentina showed substantial variability in formulation and fluoride content. Although all products complied with the regulatory upper limit of 1,500 ppm total fluoride (TF), none of them contained the minimum recommended concentration of 1,000 ppm total soluble fluoride (TSF) necessary to provide

effective anti-caries effect. These findings suggest the need to revise current regulatory policies to ensure that fluoride content is both accurately labeled and aligned with evidence-based thresholds for anticaries efficacy.

CONFLICT OF INTERESTS
The authors declare no potential conflict of interest regarding the research, authorship and/or publication of this article.

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